VOL. 31 MARCH 1955 NO. 3

BULLETIN

OF THE NEW YORK ACADEMY OF MEDICINE

Original Articles by

GENE H. STOLLERMAN

GLADYS L. HOBBY, Ph.D.

STANLEY E. DORST

MANAGEMENT OF ARTERIOSCLEROSIS

Transcription of a Panel Meeting

WILLIAM DOCK — DAVID ADLERSBERG

HOWARD A. EDER — FORREST E. KENDALL, Ph.D.

CHARLES F. WILKINSON, Jr.

SECTION ON MICROBIOLOGY
AND
NEW YORK PATHOLOGICAL SOCIETY

"an effective antirheumatic agent"*

nonhormonal anti-arthritic

BUTAZOLIDIN®

[Brand of phenylbutazone]

relieves pain · improves function · resolves inflammation

The standing of BUTAZOLIDIN among today's anti-arthritics is attested by more than 250 published reports. From this combined experience it is evident that BUTAZOLIDIN has achieved recognition as a potent agent capable of producing clinical results that compare favorably with those of the hormones.

Indications: Gouty Arthritis Rheumatoid Arthritis Psoriatic Arthritis
Rheumatoid Spondylitis Painful Shoulder Syndrome

BUTAZOLIDIN® (brand of phenylbutazone) red coated tablets of 100 mg.

*[illegible]

CIBA

GEIGY PHARMACEUTICALS

Division of Geigy Chemical Corporation, 220 Church Street, New York 13, N.Y.

Now . . . a totally new nonbarbiturate hypnotic-sedative!

for a good night's sleep with clear awakening

DORIDEN®

(glutethimide CIBA)

In most cases—

Rapid onset—15-20 minutes
Lasts 4-8 hours
No hangover

Dosage: 0.25 to 0.5 Gm. before bedtime.

Scored 0.25- and 0.5-Gm. tablets.

C I B A Summit, N. J.

2/20798

- highly soluble in both acid and alkaline body fluids, especially at pH of renal tubules
- high plasma levels rapidly attained
- no alkalinization or forcing of fluids required
- single sulfonamide - not a mixture
- no danger of secondary fungus infections
- wide antibacterial range

you prescribe
these distinctive features
when you prescribe . . .

Hoffmann - La Roche Inc • Roche Park • Nutley 10 • N.J.

GANTRISIN® -brand of sulfisoxazole (3,4-dimethyl-5-sulfanilamido-isoxazole)

The Bulletin of The New York Academy of Medicine, Vol. 31, No. 3, March, 1955. Published Monthly by The New York Academy of Medicine, 2 East 103 Street, New York 29, N. Y. Entered as second-class matter February 3, 1928, at the Post Office at New York, N. Y., under the Act of August 24, 1912. Annual subscription United States and Canada $6.50. All other countries $7.50. Single copies 75c.

CAMBRIDGE EDUCATIONAL CARDIOSCOPE

Bulletin 184

Overall Dimensions: 74" x 25" x 25"

CAMBRIDGE INSTRUMENT CO., INC.

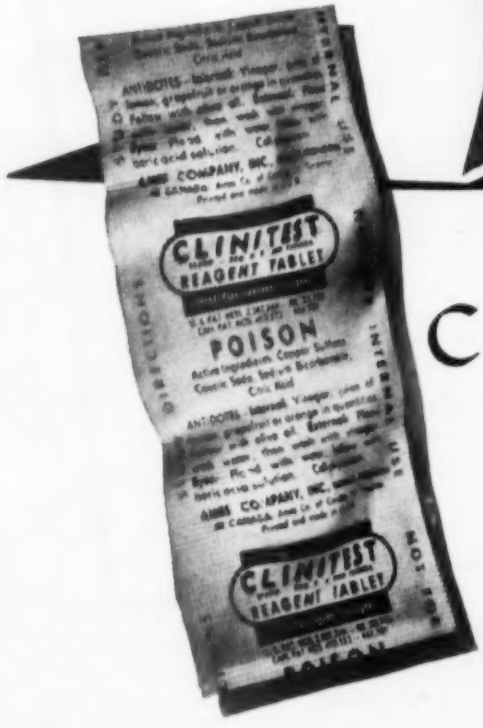
3744 Grand Central Terminal, New York, N. Y.

PIONEER MANUFACTURERS OF THE ELECTROCARDIOGRAPH

Send for
BULLETIN 184

THE CAMBRIDGE EDUCATIONAL CARDIOSCOPE is used in many prominent Medical Schools for teaching cardiology by audio-visual demonstration. The electrocardiogram, heart sounds, vectorcardiogram, EKY, BCG, intra-cardiac pressures, etc., may be individually shown on a 16" cathode ray tube having a long persistence screen. While viewing the physiological data, the related heart sounds may be heard by an entire group through individual stethophones. *With the Cambridge Educational Cardioscope, true audio-visual teaching of Cardiology is now a reality—a must for the modern teaching program.*

the last tablet as accurate as the first



sealed-in-foil CLINITEST® BRAND REAGENT TABLETS

a rapid, reliable urine-sugar test every time because every batch of *Clinitest* Sealed-in-Foil Reagent Tablets is tested for stability under conditions as exacting as a tropical rainy season—86° to 90° temperatures and 95% humidity.

Clinitest Reagent Tablets, Sealed in Foil, boxes of 24 and 500.

AMES DIAGNOSTICS
Adjuncts in Clinical Management

AMES COMPANY, INC • ELKHART, INDIANA
Ames Company of Canada, Ltd., Toronto

62754

NEW DRUG

Meratran

(pipradrol hydrochloride)

a unique central motivant with demonstrated subcortical activity subtly returns your emotionally fatigued and depressed patients to their usual level of alertness, interest and productivity[1-2]...

Meratran's action, in easily adjusted doses, is prompt– subtle – comfortable – Its effectiveness is prolonged.

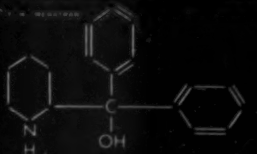
• no appreciable effect on blood pressure and respiration • restores needed sense of well-being • no tolerance or drug habituation • normal appetite undisturbed • no jitters - no apprehension • little or no insomnia • wide range of safety • no rebound letdown

Rx INFORMATION

Meratran

Indications: Emotional fatigue, unhappiness of more common type (financial worry, social stress). Situational stress or mild depression. Adjunctive therapy in certain psychoses and psychoneuroses.[3,4]

Composition: alpha-(2-piperidyl) benzhydrol hydrochloride with the following structure:



Dosage: For emotional fatigue and mild depression, *1 to 6 mg. daily. Individual patient response must be observed and daily dosage and duration of administration adjusted to patient response.*

Supplied: Small pink tablets containing: 1 mg. Meratran (pipradrol) hydrochloride.* Bottles of 100.

* Alpha-(2-piperidyl) benzhydrol hydrochloride.

1. Heath, R. C.: Discussion of paper by Howard D. Fabing (alpha-(2-piperidyl) benzhydrol hydrochloride, a new central stimulant in the treatment of blepharospasm, spasmodic torticollis and narcolepsy) presented before the American Psychiatric Association, Atlantic City, June 14, 1954. 2. Fabing, H. D., Hawkins, J. R., Moulton, J. A. L.: Clinical studies on alpha-(2-piperidyl) benzhydrol hydrochloride, a new antidepressant drug. Presented before the American Psychiatric Association, St. Louis, Missouri, May 3, 1954. 3. Fabing, H. D.: Alpha-(2-piperidyl) benzhydrol hydrochloride, a new central stimulant, in the treatment of blepharospasm, spasmodic torticollis and narcolepsy; preliminary report. (Motion Picture Demonstration). American Neurological Association, June 14, 1954. 4. Himwich, H. E.: Discussion of paper (reference 2). American Psychiatric Association, May 3, 1954.

Merrell Since 1828

THE WM. S. MERRELL COMPANY

New York • CINCINNATI • St. Thomas, Ontario

PIONEER IN MEDICINE FOR OVER 125 YEARS

We thank you, doctor, for prescribing Trevidal. Most of you who have employed this new, balanced protective antacid have continued to use it routinely. For those of you who have not yet had a chance to evaluate the unique antacid activity of Trevidal, may we suggest you write us today for a clinical trial supply.

TREVIDAL®

	EACH TABLET CONTAINS:	
Unique vegetable mucin supplies protective coat to irritated stomach lining	Regonol*‡	100 mg.
Balance of ingredients avoids constipation, diarrhea, or alkalosis	Magnesium trisilicate	150 mg.
	Aluminum hydroxide gel	90 mg.
	Calcium carbonate	105 mg.
	Magnesium carbonate	60 mg.
Binder controls and extends antacid activity	Egraine†‡	45 mg.

AVAILABLE IN BOXES OF 100 TABLETS, SPECIALLY STRIPPED FOR EASY CARRYING

*Cyamopsis tetragonoloba gum †Protein binder from oat ‡Trade Marks

Organon INC. • ORANGE, N. J.

Preceptin
simple, effective conception control
Ortho
PRECEPTIN
vaginal gel

3 STEPS

for accurate determination of thyroid function

using radioiodine (I^{131})

The "MEDIAC" makes measurements of thyroid activity a simple, straightforward procedure, requires no computations—takes but a few minutes. AEC requirements can be *easily* met by any physician, and the equipment cost is lower than most doctors think. The evaluation is more accurate than the BMR test, easier than the serum protein bound iodine level, is rapidly becoming routine in hospitals, clinics and the doctor's office.

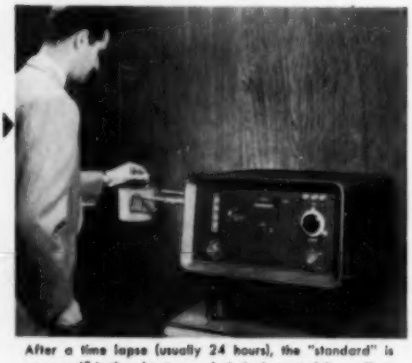
Write today for Catalog "O" with complete details on the "MEDIAC" and other medical instruments for use with radioisotopes.

Nuclear Instrument and Chemical Corporation,
259 West Erie Street, Chicago 10, Illinois

nuclear-chicago

CHICAGO · NEW YORK · LOS ANGELES
DALLAS · HOUSTON · SILVER SPRING, MD.

Patient is given a radioiodine capsule or solution by mouth. An identical dose is set aside as a "standard."



After a time lapse (usually 24 hours), the "standard" is measured* in the phantom neck. A single control then adjusts the "MEDIAC" for the patient.

Phantom is removed and activity in patient's thyroid is measured.* Uptake of radioiodine is directly related to thyroid function and shown as *a per cent* of the administered dose.

*Maximum time involved—2 minutes.

first in advantages for low-salt therapy

DIASAL®

salt substitute of choice

for congestive failure
essential hypertension
obesity
and whenever low salt is indicated

TASTE POUR-QUALITY APPEARANCE STABILITY	help keep patients on low-sodium diets
FREEDOM FROM UNPLEASANT AFTERTASTE	Keeps food attractive— prevents nutritional deficiencies
OPTIMAL POTASSIUM CHLORIDE CONTENT	avoids borderline hypopotassemia—makes DIASAL "...the product of choice for this purpose."*

DIASAL contains only potassium chloride, glutamic acid and inert ingredients...no sodium, lithium, or ammonium... and it is safe for prolonged use, both at the table and in cooking.

packaging: available in 2-ounce shakers and 8-ounce bottles. Send for liberal supplies of tasting samples and low-sodium diet sheets for your patients.

*Fremont, R. E.; Rimmerman, A. B., and Shaftel, H. E.: Postgrad. Med. *10*:216, 1951.

E. FOUGERA & CO., INC. 75 Varick St., N. Y. 13, N. Y.

'Sulfa-Neolin'

(BENZATHINE PENICILLIN—G WITH SULFONAMIDES, LILLY)

chocolate-mint-flavored suspension for the treatment of mixed infections caused by penicillin-susceptible and/or sulfonamide-sensitive organisms

The twofold antibacterial action of 'Sulfa-Neolin' is particularly effective in mixed infections. The combined action of penicillin and the sulfonamides is also of value in combating relatively resistant organisms. Since the mode of attack of penicillin differs from that of the sulfonamides, it is more difficult for an organism to develop resistance to the combination than to either drug used alone. The tasty chocolate-mint flavor of 'Sulfa-Neolin' quickly wins youngsters' approval. They'll like it!

Each 5 cc. (approximately 1 teaspoonful) contain sulfadiazine, merazine, methazine, 0.167 Gm. each, and benzathine penicillin—G, 300,000 units. 'Sulfa-Neolin' is stable at room temperature for two years.

Dosage: Average dose is 1 teaspoonful four times a day.

Suspension 'Sulfa-Neolin' is supplied in 60-cc. bottles (M-120).

ELI LILLY AND COMPANY • INDIANAPOLIS 6, INDIANA, U.S.A.

SECOND SERIES MARCH 1955 VOL. 31, NO. 3

BULLETIN OF THE NEW YORK ACADEMY OF MEDICINE

CONTENTS

The Prevention of Rheumatic Fever by the Use of Antibiotics 165
Gene H. Stollerman

The Current Status of the Development of Antimicrobial Agents 181
Gladys L. Hobby, Ph.D.

Current Concepts in the Management of Arteriosclerosis 198
Transcription of a Panel Meeting on Therapeutics
William Dock, David Adlersberg, Howard A. Eder, Forrest E. Kendall, Ph.D. and Charles F. Wilkinson, Jr.

Our Heritage from William Osler 220
Stanley E. Dorst

Section on Microbiology and New York Pathological Society 231

AUTHORS ALONE ARE RESPONSIBLE FOR OPINIONS EXPRESSED IN THEIR CONTRIBUTIONS

ROBERT L. CRAIG, *Editor*

Published Monthly by THE NEW YORK ACADEMY OF MEDICINE
2 East 103 Street, New York 29, N. Y.

Annual subscription United States and Canada $6.50. All other countries $7.50. Single copies 75c.
ADVERTISING OFFICE • 30 ROCKEFELLER PLAZA, N. Y. C. 20 • CIRCLE 7-7706
Copyright, 1955 by The New York Academy of Medicine

OFFICERS AND STAFF OF THE ACADEMY

1955

President

EDWARD J. DONOVAN

Vice-Presidents

JEROME P. WEBSTER — J. BURNS AMBERSON — ROBERT L. LEVY

Treasurer

SHEPARD KRECH

Recording Secretary

FREDERICK H. WILKE

Trustees

GEORGE BAEHR	°SHEPARD KRECH	HENRY ALSOP RILEY
BRADLEY L. COLEY	ASA L. LINCOLN	BENJAMIN P. WATSON
CONDICT W. CUTLER, JR.	WILLIAM F. MACFEE	ORRIN S. WIGHTMAN
°EDWARD J. DONOVAN	HAROLD R. MIXSELL	°FREDERICK H. WILKE
	WILLIAM BARCLAY PARSONS	

Council

The President — The Vice-Presidents — The Trustees
The Treasurer — The Recording Secretary
The Chairmen of Standing Committees

Director

HOWARD REID CRAIG

Librarian

JANET DOE

Executive Secretary
Committee on Public Health

HARRY D. KRUSE

Executive Secretary
Committee on Medical Education

ROBERT L. CRAIG

Executive Secretary
Committee on Medical Information

IAGO GALDSTON

Comptroller

ARTHUR A. EBERLE

Legal Counsel

JOHN W. DAVIS, ESQ.

Library Consultants

B. W. WEINBERGER, CURT PROSKAUER

EDITORIAL BOARD

JEROME P. WEBSTER, *Chairman*	JANET DOE
DAVID P. BARR	JOHN G. KIDD
MARGARET BEVANS	ROBERT F. LOEB
WILLIAM DOCK	LOUIS M. ROUSSELOT

ROBERT L. CRAIG, *Secretary and Editor*

° Ex-officio

BULLETIN OF
THE NEW YORK ACADEMY
OF MEDICINE

VOL. 31, NO. 3 MARCH 1955

THE PREVENTION OF RHEUMATIC FEVER BY THE USE OF ANTIBIOTICS*

GENE H. STOLLERMAN

Medical Director, Irvington House, Instructor in Medicine
New York University College of Medicine

It is becoming increasingly evident that the proper treatment and control of group A streptococcal infection will markedly reduce the incidence, morbidity and mortality of rheumatic fever. Although the pathogenesis of the disease remains obscure, the group A streptococcus has been firmly established as the inciting agent of first attacks and recurrences of rheumatic fever.[1-4] The prevention of rheumatic fever depends, therefore, upon a thorough understanding of the chemoprophylaxis and chemotherapy of streptococcal infections. An intimate knowledge of the relationship of the streptococcus to the natural history of rheumatic fever is necessary to appreciate the potentialities, as well as the limitations, of this method of prophylaxis.

The Influence of Chemoprophylaxis of Streptococcal Infection upon the Natural History of Rheumatic Fever: It has been possible to demonstrate serologic evidence of recent streptococcal infection in almost every patient with acute rheumatic fever.[5] In a recent study at Irvington

* Presented at the 27th Graduate Fortnight of The New York Academy of Medicine, October 22, 1954.
From Irvington House, Irvington-on-Hudson, N. Y. and the Department of Medicine, N.Y.U. College of Medicine, New York.

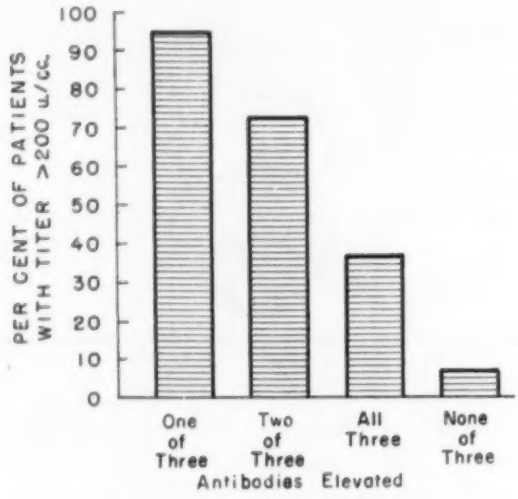


Fig. 1—Incidence of elevated titers of antistreptolysin O, antihyaluronidase and antistreptokinase in the serum of 88 patients observed within two months of the onset of acute rheumatic fever.[5]

House it was demonstrated that the titer of antistreptolysin O is elevated above 200 units per cc. in about 80 per cent of patients who were observed within two months of the onset of an acute rheumatic attack. When the titer of streptococcal antihyaluronidase was also determined on the same sera, an elevation of *either* antibody was demonstrable in 90 per cent of patients. When a third specific streptococcal antibody, antistreptokinase, was assayed, 95 per cent of the group showed significant elevation of at least one antibody (Fig. 1). This percentage approaches 100, the earlier in the rheumatic attack the patient is studied.[6]

Once the streptococcus initiates a rheumatic episode the subsequent course of the attack is capricious and unpredictable, even though further streptococcal infection is prevented. It has been commonly observed, however, that if new streptococcal infection occurs while the rheumatic process is still active, it may provoke violent and even fatal exacerbation of rheumatic fever. The protection of the patient from new infection, and the prompt eradication of streptococci from his throat at the inception of the rheumatic attack, is important and may be one of the factors contributing to the declining mortality and severity of

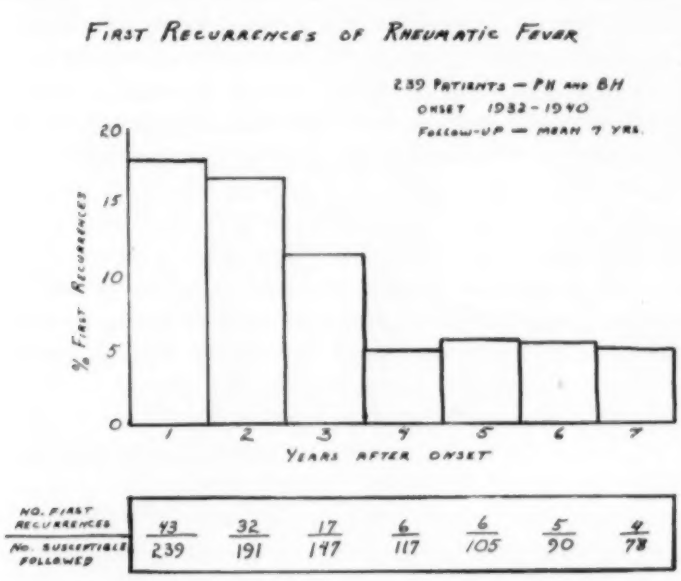


Fig. 2—Annual incidence of first recurrences of rheumatic fever among 239 patients followed at Presbyterian and Babies Hospitals, N.Y.C., for a mean period of seven years after an attack of rheumatic fever. Onset of acute rheumatic fever occurred between 1932 and 1940. (Fischel, E. E., unpublished data.)

acute rheumatic fever and to the increasing percentage of patients who recover from initial attacks of the disease without permanent cardiac damage.

When rheumatic fever becomes completely quiescent, and remains so for a period of at least two months, relapses occur rarely, if ever, without new streptococcal infection.[5] The course of rheumatic fever was studied in 580 patients hospitalized at Irvington House where streptococcal infection was controlled. "Spontaneous relapses" (those not associated with new streptococcal infection) occurred invariably within two months of discontinuing aspirin, cortisone or ACTH therapy. Relapses of rheumatic fever which appeared *after* two months of apparent complete quiescence of the disease were always associated with bacteriologic and serologic evidence of new streptococcal infection.

It follows from the results of these and other studies that recurrences of rheumatic fever that are not relapses of persistent rheumatic activity are preventable by complete protection of the rheumatic subject from

new streptococcal infection. The risk of recurrences of rheumatic fever to the rheumatic subject in the era before continuous antibiotic prophylaxis was employed was great. From 60 to 75 per cent of patients who had one episode of rheumatic fever suffered a recurrence.[6] The pattern of recurrence rates is shown by the data of Fischel (Fig. 2) who observed the subsequent course of rheumatic attacks in 239 subjects followed for an average period of seven years at the Babies and the Presbyterian Hospitals in New York City. These patients suffered an initial attack in the years between 1932 and 1940. The greatest number of recurrences appeared in the first three years following an episode of rheumatic fever. Thereafter the recurrence rate fell off sharply, approaching a rather constant value after the fifth year.

In contrast to this experience of a little more than a decade ago is the rate of recurrences among rheumatic subjects now receiving continuous chemoprophylaxis. In the past two years children attending the Irvington House out-patient prophylaxis clinic and the Childrens' Cardiac Clinic at Bellevue Hospital have received either sulfonamides or penicillin orally every day, or one injection of benzathine penicillin intramuscularly every month. There have been but seven recurrences (all in the group receiving oral prophylaxis) in a highly susceptible group representing 543 patient-years of observation. This represents a recurrence rate of 1.2 per cent compared with the yearly rate of about 15 per cent in Fischel's series of untreated patients during a previous era. The reduction of over 90 per cent in the incidence of recurrences is in close agreement with a compilation of controlled studies which have been carried out during the past fifteen years and which have been summarized in several recent reviews.[7, 8]

There remains little doubt, therefore, that overt rheumatic recurrences can be prevented. Of great importance, however, is the question as to what extent antistreptococcal prophylaxis will alter the natural history of rheumatic heart disease. In a twenty year study during the "preprophylaxis" era, Bland and Jones[9] noted the delayed appearance of rheumatic heart disease in almost half of the patients who recovered initially from an attack of rheumatic fever without apparent cardiac damage. The implication of this study is that either subclinical valvular sclerosis progresses inexorably following an initial attack or that the late appearance of such lesions is the result of repeated attacks of rheumatic fever, often subclinical, and caused by intercurrent streptococcal infec-

tion. Careful prophylaxis maintained over a period of years, and detailed immunologic studies to detect subclinical streptococcal infection, should determine whether the delayed appearance of rheumatic heart disease can be prevented. Preliminary observations made for a period of three years have been encouraging[10] but do not yet provide conclusive answers.

The data of Bland and Jones[9] also indicate that a relatively high percentage (about one-third) of patients who sustain rheumatic heart disease following an attack of rheumatic fever ultimately heal their lesions partially or completely. It is probable that the prognosis for the young rheumatic cardiac will be further improved with the use of continuous prophylaxis. Indeed, the longevity of the rheumatic cardiac may depend to a considerable extent upon his protection from renewed bouts of carditis. Unfortunately, there is as yet no known way to terminate those attacks of rheumatic fever which enter a chronic phase and result in severe cardiac damage despite complete protection from intercurrent streptococcal infection. Fortunately, this happens in less than 5 per cent of attacks of acute rheumatic fever.[5] The finding of Aschoff bodies in the auricular appendage of some patients with advanced mitral stenosis who have undergone mitral commissurotomy attests to the chronicity of the rheumatic process in such patients. Again, it should be noted that such patients constitute a small percentage of all rheumatic cardiacs. It is far more common to find rheumatic heart disease at postmortem examination *without* microscopic evidence of active lesions of rheumatic fever. It remains for future studies to determine whether careful protracted prophylaxis in the rheumatic subject following an initial attack of rheumatic fever will reduce the frequency with which rheumatic valvular disease develops.

General Principles of Prophylaxis for the Rheumatic Subject: In view of the above considerations, the general principles governing the use of continuous chemoprophylaxis which have been recommended by the Committee on Prevention of Rheumatic Fever of the American Heart Association[11] deserve emphasis.

1. It is generally agreed that prophylaxis for the highly susceptible rheumatic subject must be *continuous*. For such patients it is unsafe to await clinical signs of upper respiratory infection before initiating antibiotic prophylaxis.

2. *Selection of patients* should include all children and adolescents who have had an attack of rheumatic fever and all adults who have

had an attack within five years. In our opinion all patients who have definite rheumatic heart disease should be included, regardless of the interval since the last known attack. Patients whose clinical manifestations of rheumatic fever meet the diagnostic criteria of Jones[12] present no problem of selection. The frequency with which rheumatic fever begins as an ill-defined clinical syndrome, however, imposes an important limitation upon this method of prophylaxis. The decision to start prophylaxis for a period of years is a serious one and involves the possibility of causing psychic trauma and even invalidism unnecessarily in individuals who are not really rheumatic. At the risk of failing to recognize some rheumatic subjects, it is best to reserve prophylaxis for those whose diagnosis is firmly established.

3. *Prophylaxis should begin* as soon as rheumatic fever has been diagnosed and after a course of penicillin has been given which is adequate to eradicate group A streptococci (see below). The absence of clinical signs of pharyngitis and the failure to culture group A streptococci from the nose or throat may not always exclude the presence of these organisms deeper in the tissues, particularly in the presence of enlarged tonsils.

4. The *duration* of continuous prophylaxis is a matter to be determined to some extent by judgment and individual circumstances. It would be wise to protect the rheumatic patient as long as the risk of streptococcal exposure remains high and certainly for a minimum period of five years after an attack, when the recurrence rate is greatest. It is not yet known whether the attack rate of rheumatic fever following streptococcal infection will be diminished when prophylaxis is maintained for several years. The incidence of rheumatic recurrences following streptococcal infection among patients recently recovered from a previous rheumatic episode is as high as 50 per cent. The attack rate of rheumatic fever following streptococcal epidemics in the general population is somewhat less than 3 per cent. It will be of considerable importance to determine whether the high attack rate of rheumatic fever in the rheumatic subject is an acquired characteristic which may eventually be lost after several years of prophylaxis.

5. *Seasonal variation* in the incidence of rheumatic fever does not justify discontinuing prophylaxis during the summer months. As a matter of fact, the incidence of streptococcal infection and rheumatic fever is actually lowest in the New York area in the late autumn months.

TABLE I—METHODS OF CONTINUOUS CHEMOPROPHYLAXIS

	Sulfadiazine	*Oral Penicillin*	*Repository Benzathine Penicillin*
ADVANTAGES	1. Easily administered 2. Well absorbed 3. Inexpensive 4. Established effectiveness	1. Bactericidal 2. Serious toxicity rare 3. No resistant streptococci	1. "Break" in prophylaxis less likely 2. Single dose usually eliminates carrier state 3. Economical 4. Close patient contact 5. Other advantages of penicillin (See Oral Penicillin)
DISADVANTAGES	1. Frequent breaks in routine 2. Risk of serious toxicity 3. Resistant streptococci 4. Bacteriostatic	1. Frequent breaks in routine 2. Irregular absorption 3. Costly 4. Minimum effective dose not well established	1. Requires injection 2. Moderate local soreness
RECOMMENDED DOSE	1.0 Gm./day	200,000-250,000 units once or twice daily	1,200,000 units I.M. once monthly
TOXICITY	Skin eruptions—0.5% Blood reactions—0.01%	Urticaria Angioneurosis—0.3-0.7% "Serum type sickness" Anaphylaxis—rare "Periarteritis"	Urticaria Angioneurosis—1-2% "Serum type sickness" Anaphylaxis—? "Periarteritis"

Sporadic streptococcal infection occurs throughout the year, however, and therefore prophylaxis should be maintained the year round.

Choice of Antibiotic and Mode of Administration: Many antibiotics are effective against group A streptococci. This discussion is confined to an evaluation of three methods that have been employed most extensively for the prevention of rheumatic recurrences (Table I).

Sulfonamides: During the past fifteen years many controlled independent studies[7, 8] and a vast military experience during World War II[13] have demonstrated conclusively that the daily oral administration of small doses of sulfonamides will reduce the incidence of rheumatic recurrences by at least 85 per cent. Sulfadiazine is generally considered the drug of choice, although some of the newer, more soluble sulfonamides may be equally effective.[14] Sulfadiazine is inexpensive, easily administered, well absorbed from the gastrointestinal tract and of established effectiveness.

There are several disadvantages to this method. Frequent breaks in the routine occur because patients are apt to forget to take the medication daily. There is no practical way to determine whether the occurrence of a streptococcal "breakthrough" is due to failure of the drug to protect or to a lapse in the regimen. Another disadvantage is that the action of sulfonamides is bacteriostatic rather than bactericidal. The sulfonamides do not eradicate the streptococcal carrier state. In addition, sulfonamide resistant streptococci have appeared during mass prophylaxis programs conducted in the Armed Forces and occasionally among civilian populations.[13, 15] Such strains do not constitute a serious threat, however, because of the availability of other effective antibiotics. Finally, the risk of occasional serious toxicity, particularly granulocytopenia, is sufficient to require routine white blood cell counts, at least during the first several weeks of the prophylactic regimen.

The daily dose of sulfadiazine recommended is 1.0 gm. There is less likelihood of doses being omitted when this is administered once daily. Toxic reactions to such small doses of sulfadiazine are relatively infrequent. The incidence of mild reactions such as skin rashes is 0.5 per cent. More serious reactions such as agranulocytosis and exfoliative dermatitis occur in about one in 10,000 patients.[13]

Oral Penicillin: There have been relatively few extensive, controlled studies in which penicillin has been administered in small daily oral doses to prevent rheumatic recurrences. Those that have been reported indi-

cate, however, that this method should be at least as effective as the employment of sulfonamides.[8] Extensive studies have been conducted recently in the Armed Forces which indicate that daily oral doses of 50,000 to 100,000 units of penicillin are highly effective during epidemics of streptococcal pharyngitis in protecting troops from both infection and infestation.[16] It is not yet certain that such small doses will protect the highly susceptible young rheumatic subject from recurrence.

Penicillin has several advantages over sulfonamides. It is a more effective antibiotic against group A streptococci and is bactericidal rather than bacteriostatic in its action. In adequate doses it will eradicate the streptococcal carrier state (see below). Serious toxic reactions are quite rare. Penicillin-resistant group A streptococci have not been encountered despite the extensive use of this compound in mass prophylaxis studies.[16]

There are, however, some disadvantages to this form of prophylaxis. As with the use of sulfonamides, frequent breaks in the prophylactic regimen may occur. The absorption of penicillin from the gastrointestinal tract is irregular. The cost of relatively large oral doses is still high. The minimum effective daily oral dose has not yet been established. It has been shown that daily oral doses of 500,000 units are highly effective in curing streptococcal pharyngitis, eradicating the streptococcal carrier state and preventing new infection and infestation.[17,18] If the encouraging results with the use of daily doses of 100,000 units for the protection of normal adults from streptococcal infection can be applied with equal success to the protection of the rheumatic subject, the cost of this method may be reduced considerably. At the present time it is suggested that oral penicillin should be given in doses of at least 200,000 to 250,000 units once or twice daily until more information on the use of smaller doses is available.

There is considerable confusion in the literature with regard to the true incidence of penicillin hypersensitivity. Such confusion stems most often from the lack of controlled observations and from the absence of definite criteria for the diagnosis of penicillin hypersensitivity reactions. Such factors as the size of the dose of penicillin employed, the age of the patient, previous contact with penicillin and the route and duration of administration may also influence the incidence of reported toxic reactions. In general, the incidence of reactions from the administration of small, daily oral doses of penicillin (100,000 units or less) to large numbers of troops during a period of several months has been between 0.3 to 0.7 per cent. When placebos of sodium bicarbonate were substituted

for penicillin and administered to a large control group without the knowledge of the medical observers, the incidence of "penicillin reactions" was reported to be 0.2 per cent! The incidence of reactions in the penicillin treated group was 0.3 per cent.[18]

Most of the penicillin reactions encountered in these studies, and in the more limited studies conducted with children and adolescents, have been mild and transient, and usually consist of generalized urticaria. In most instances if penicillin is discontinued for a few days it can be restarted without causing further reactions.[19] When reactions are more severe and associated with angioneurotic edema, penicillin should be discontinued. Even these reactions, however, do not necessarily indicate permanent sensitization to penicillin.

Repository Penicillin (Benzathine Penicillin): The major disadvantage of daily oral prophylactic medication is that ambulatory patients are apt to forget to take the tablets regularly. Recently, a new repository penicillin compound, benzathine penicillin (N, N' dibenzylethylenediamine dipenicillin G) has been developed. The injection of 600,000 units of this compound provides low serum levels of penicillin for twelve to fourteen days. A single injection of 1.2 million units results in the appearance of detectable penicillin levels in blood for three to four weeks.[20]

Monthly injections of 1.2 million units of benzathine penicillin have proven very effective in preventing streptococcal infections and recurrence of rheumatic fever. During a recent two year study there were no recurrences of rheumatic fever among 145 patients attending the Irvington House Prophylaxis Clinic monthly to receive injections of benzathine penicillin. These patients had recently recovered from attacks of rheumatic fever and resided in low-income housing areas in New York City where the incidence of streptococcal infection is known to be high. Of 2,716 throat cultures made routinely, three were positive for group A streptococci. A significant rise in antistreptolysin O titer, which was determined monthly, was noted in but three instances.[21]

Similar results were obtained in an independent study of ninety-six rheumatic fever patients who received 1.2 million units of benzathine penicillin monthly during a period of fourteen months.[22]

Advantages of this form of continuous prophylaxis are several. A break in the regimen is less likely to occur than when medication is administered daily. A single dose administered intramuscularly is highly

effective in curing streptococcal pharyngitis and in eradicating the streptococcus carrier state (see below). This is not true of small oral doses of penicillin or sulfonamides. Single injections of benzathine penicillin are economical. The cost of 12 intramuscular doses is similar to, or less than that of sulfadiazine administered daily in doses of 1.0 gm. for one year. The method affords close patient contact and supervision.

Its major disadvantages relate principally to the inconvenience and pain of injections. The site of injection tends to be somewhat more painful with the use of this compound than with procaine penicillin. Severe local reactions resulting in abscess formation have not been observed. Occasionally local soreness is attended by mild fever lasting a day or two.

Hypersensitivity reactions so far have not been more frequent or more severe than those observed with the parenteral use of other forms of penicillin. During the past three years, 6,624 injections have been administered to 553 patients with rheumatic fever on the wards and in the out-patient department at Irvington House for periods of ten to twenty-one months. Six patients developed transient urticaria which lasted for three to four days and subsided despite the persistence of penicillin in the blood for several weeks thereafter. One patient developed angioneurotic edema and penicillin was permanently withdrawn, although the reaction lasted but a few days. Eight patients developed non-specific rashes which were evanescent, did not recur with continued therapy and could not be definitely attributed to penicillin allergy. Similar experience has been observed by others who have treated large numbers of children with this compound.[22]

In adults, the incidence of what appears to be penicillin hypersensitivity reactions has been reported as 2.3 per cent in one study when single injections of 1.2 million or 600,000 units were administered to 257 subjects.[23] This is comparable to the incidence of similar reactions with the use of procaine penicillin or crystalline penicillin G. Carefully controlled studies on the relative frequency of hypersensitivity reactions to various types of penicillin administered parenterally are not available. So far it would appear that the parenteral use of benzathine penicillin has not been attended by a higher incidence of hypersensitivity than has been encountered with the parenteral use of other penicillin compounds.

The choice of one of the three types of prophylactic medication

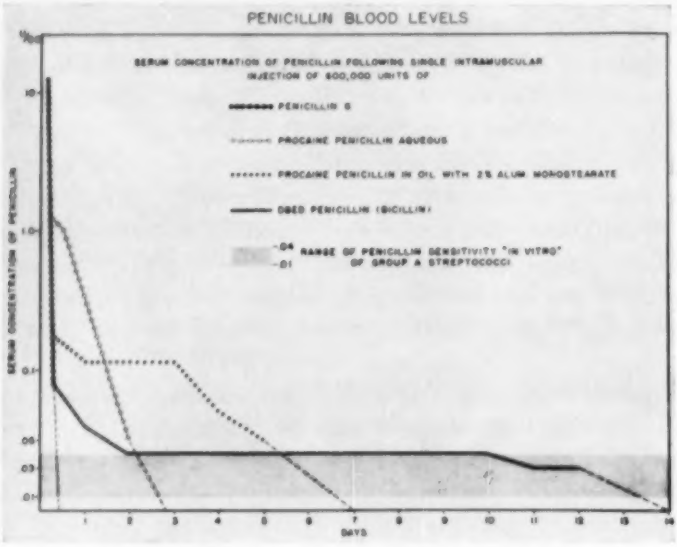

PENICILLIN BLOOD LEVELS
SERUM CONCENTRATION OF PENICILLIN FOLLOWING SINGLE INTRAMUSCULAR INJECTION OF 600,000 UNITS OF
PENICILLIN G
PROCAINE PENICILLIN AQUEOUS
PROCAINE PENICILLIN IN OIL WITH 2% ALUM MONOSTEARATE
OBED PENICILLIN (BICILLIN)
.04 RANGE OF PENICILLIN SENSITIVITY "IN VITRO"
.01 OF GROUP A STREPTOCOCCI
SERUM CONCENTRATION OF PENICILLIN
DAYS

Fig. 3—Stollerman, G. H.[27] Prevention of rheumatic fever, *American Practitioner and Digest of Treatment*, 5:589, 1954. Reproduced with permission of the publisher, J. B. Lippincott Company.

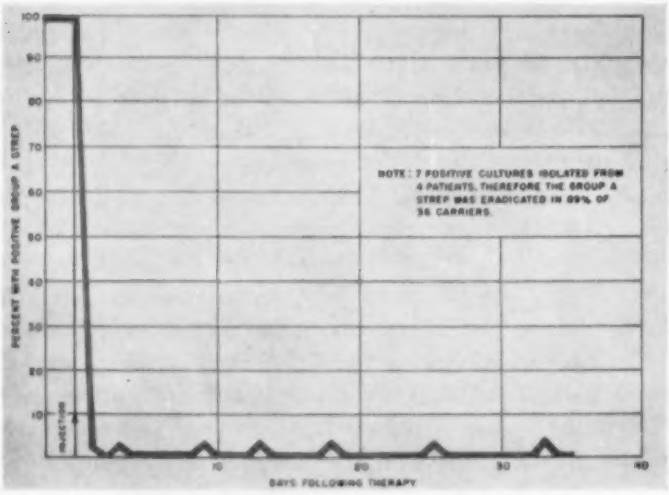

PERCENT WITH POSITIVE GROUP A STREP
INJECTION
NOTE: 7 POSITIVE CULTURES ISOLATED FROM 4 PATIENTS. THEREFORE THE GROUP A STREP WAS ERADICATED IN 89% OF 36 CARRIERS.
DAYS FOLLOWING THERAPY

Fig. 4—Effect of single intramuscular injection of benzathine penicillin, 600,000 to 1,200,000 units, upon group A streptococcal carriers. Throat cultures made daily for one week following injection and weekly thereafter.

discussed above will vary with individual considerations. Some patients may be relied upon to take tablets daily with regularity. For others it may be safer and simpler to administer monthly injections. Some may find injections too objectionable over prolonged periods of time. The choice of a particular prophylaxis regimen is less important than an appreciation, on the part of both patient and physician, of the importance of strict adherence to *some* effective form of prophylaxis.

Prevention of Initial Attacks of Rheumatic Fever

Initial attacks of rheumatic fever may be prevented by the prompt and adequate penicillin treatment of streptococcal infection.[24-26] Certain chemotherapeutic principles upon which such prevention is based have been firmly established, primarily as a result of the admirably controlled large scale studies conducted at the Streptococcus Disease Laboratory at Francis E. Warren Air Force Base.[25, 26]

General Principles: To prevent the complication of subsequent rheumatic fever, group A streptococci must be eradicated completely from the nose and throat of the patient with streptococcal pharyngitis. Clinical cure of the signs and symptoms of the streptococcal infection is inadequate if organisms persist after treatment. Sulfonamides, which are bacteriostatic rather than bactericidal agents, do not eradicate the streptococcal carrier state. Penicillin, although a powerful bactericidal agent against group A streptococci, must be administered well beyond the period of clinical cure to eradicate these organisms from the throat completely.

The therapeutic regimen required to achieve bacteriologic cure and eradication of the streptococcal carrier state with penicillin has been clearly established.[17, 18] If penicillin is administered orally, a minimum daily dose of 500,000 units must be given for ten days. Larger doses given for shorter periods are less effective.[17] If penicillin is administered parenterally any schedule may be employed which maintains effective blood concentrations of penicillin for a ten day period. The average serum levels resulting from a single injection of different forms of penicillin are shown in Fig. 3.[27] Group A streptococci are exquisitely sensitive to penicillin (almost invariably less than 0.04 units/ml. in vitro). The blood concentration of penicillin need not be high to achieve a maximum killing effect. Once such levels have been provided, the efficiency of chemotherapy is a function of their *duration*.[28]

Thus, a single large dose of crystalline penicillin G which provides very high levels for but a few hours is ineffective. Similarly, a single dose of procaine penicillin G, aqueous, or in oil with 2 per cent aluminum monostearate, will provide relatively high blood levels for several days and will usually produce clinical cure of the pharyngitis but will not effectively eradicate the streptococcal carrier state. A single injection of 600,000 to 1.2 million units of benzathine penicillin will, however, achieve bacteriologic as well as clinical cure.[20, 23]

One of the following schedules is suggested for the treatment of streptococcal pharyngitis:

1. One injection of 600,000 to 1.2 million units of benzathine penicillin.
2. 600,000 units of procaine penicillin in oil with 2 per cent monostearate given every other day for 3 or 4 doses.
3. 300,000 units of aqueous procaine penicillin daily for ten days.
4. At least 500,000 units given by mouth daily for ten days.

The treatment of streptococcal infection with Aureomycin also reduces the incidence of rheumatic sequelae. It is somewhat less effective than penicillin.[29] The dose of Aureomycin recommended is about 2 grams daily for seven days. Its use is preferred for patients who are sensitive to penicillin.

The earlier in the course of streptococcal pharyngitis that penicillin is instituted the more effective is prevention of subsequent rheumatic fever. Optimum results are obtained when treatment is initiated within forty-eight hours of symptoms of sore throat. There is some recent evidence to suggest, however, that treatment instituted even as late as nine days after the onset of symptoms of sore throat may exert some favorable prophylactic effect upon the incidence of rheumatic sequelae.[30]

It is quite apparent that the major limitation of the chemotherapeutic approach to the prevention of rheumatic fever is the difficulty of clinical identification of streptococcal sore throat. It is important to avoid the promiscuous and unnecessary administration of penicillin to patients with viral upper respiratory infections. Simple coryza, hoarseness, and tracheitis are rarely due to streptococci. The syndrome of sudden onset of fever, sore throat, "beefy" redness of the pharynx and pharyngeal exudate suggests the diagnosis. Evidence of cervical lymphadenitis and the presence of leukocytosis lend further support to it. With experience a proper diagnosis of streptococcal pharyngitis can be made on

clinical criteria alone with 70 per cent accuracy.[31]

Because the preceding streptococcal infection may be inapparent in at least 38 per cent of patients who develop rheumatic fever[32] it is obvious that the chemotherapeutic approach will prevent, at best, little more than half of the total cases. Proper diagnosis and treatment may reduce prevalence of streptococcal infections and this could be reflected in a significant decline in the incidence of rheumatic fever.

It is obvious that the use of antibiotics is far from the ideal prophylaxis of rheumatic fever. Pending more knowledge and a better approach, however, the incidence and morbidity of the disease can be reduced significantly by an appreciation on the part of physicians and the general public of the importance of early diagnosis and proper therapy of streptococcal disease and upon the diligent protection of rheumatic subjects from streptococcal infection.

REFERENCES

1. Rantz, L. A. *The prevention of rheumatic fever.* Springfield, Ill., Charles C. Thomas, 1952.
2. Swift, H. F. The etiology of rheumatic fever, *Ann. intern. Med. 31:*715-38, 1949.
3. Stollerman, G. H. Rheumatic fever, in Holt, L. E., Jr. and McIntosh, R. *Holt Pediatrics,* New York, Appleton-Century Crofts, Inc., 1953, pp. 823-840.
4. Rheumatism and arthritis; review of American and English literature of recent years (Tenth Rheumatism Review), edited by W. D. Robinson, *Ann. intern. Med. 39:*498-618; 757-906, 1953.
5. Stollerman, G. H., Lewis, A., Schultz, I. and Taranta, A. The relationship of the immune response to group A streptococci to the course of acute, chronic and recurrent rheumatic fever. Read before the Second World Congress of Cardiology and 27th Scientific Session of the American Heart Association, Washington, D.C. Sept. 15, 1954.
6. Hansen, A. E. Rheumatic recrudescences, diagnosis and prevention, *J. Pediat. 28:*296-308, 1946.
7. Denny, F. W., Jr. The prophylaxis of streptococcal infections, in *Streptococcal infections,* edited by Maclyn McCarty, New York, Columbia Univ. Press, 1954. pp. 176-196.
8. Stollerman, G. H. The use of antibiotics in the prevention of rheumatic fever, *Amer. J. Med. 17:*757-67, 1954.
9. Bland, E. F. and Jones, T. D. Rheumatic heart disease; a twenty year report on 1000 patients followed since childhood, *Circulation 4:*836-43, 1951.
10. Stollerman, G. H. The use of benzathine penicillin for prevention of rheumatic fever, *Bull. Rheum. Dis. 5:*79-80, 1954.
11. Committee on Prevention of Rheumatic Fever, Council on Rheumatic Fever and Congenital Heart Disease of the American Heart Association. Report, *Mod. Concepts Cardiov. Dis. 22:*158-61, 1953.
12. Jones, T. D. The diagnosis of rheumatic fever, *J.A.M.A. 126:*481-84, 1944.
13. Coburn, A. F. and Young, D. C. *The epidemiology of hemolytic streptococcus during World War II in the United States.* Baltimore, Williams and Wilkins Company, 1949.
14. Bundy, W. E., McCue, C. M. and Porter, R. R. The control of rheumatic fever recurrences with sulfadiazine and gantrisin, *J. Pediat. 41:*320-27, 1952.
15. Johnson, R. D. and Hartman, T. L. Sulfadiazine resistant streptococcal infections in civilian community, *J. clin.*

Invest. 26:325-28, 1947.

16. Seal, J. R., Mogabgab, W. J., Friow, G. J. and Banta, J. E. Penicillin prophylaxis of epidemic streptococcal infections; the epidemic and effects of prophylaxis on the clinical manifestations of acute streptococcal and non-streptococcal respiratory infections; the effects of small and large doses of oral penicillin on epidemic streptococcal infections and on carriers of Group A. streptococci, *J. Lab. clin. Med. 44*:752-53; 831-59, 1954.
17. Wannamaker, L. W., Denny, F. W., Perry, W. D., Rammelkamp, C. H., Jr., Eckhardt, G. C., Houser, H. B. and Hahn, E. O. The effect of penicillin prophylaxis on streptococcal disease rates and the carrier state, *New Engl. J. Med. 249*:1-7, 1953.
18. Bernstein, S. H., Feldman, H. A., Harper, O. F., Klingensmith, W. H. and Cantor, J. A. Observations in Air Force recruits of streptococcal disease and their control with orally administered penicillin, *J. Lab. clin. Med. 44*: 1-13, 1954.
19. Kuttner, A. G. Prevention of rheumatic fever and rheumatic heart disease, *Postgrad. Med. 14*:429-32, 1953.
20. Stollerman, G. H. and Rusoff, J. H. Prophylaxis against group A streptococcal infections in rheumatic fever patients; use of new repository penicillin, *J.A.M.A. 150*:1571-76, 1952.
21. Stollerman, G. H., Rusoff, J. H. and Hirschfeld, I. Prophylaxis against group A streptococci in rheumatic fever patients by the use of single monthly injections of benzathine penicillin, presented at the Scientific Session of the 18th Annual Meeting of the American Rheumatism Assoc., San Francisco, Cal. June 19, 1954, also in *Antibiotics Annual 1953-54*, New York, Medical Encyclopedia Inc., 1953, pp. 114-118.
22. Diehl, A. M., Hamilton, T. R., Keeling, I. C. and May, J. S. Long-acting repository penicillin in the prophylaxis of recurrent rheumatic fever, *J. Amer. med. Assoc. 155*:1466-70, 1954.
23. Chamovitz, R., Catanzaro, F. J., Stetson, C. A. and Rammelkamp, C. H., Jr. Prevention of rheumatic fever by treatment of previous streptococcal infection; evaluation of benzathine penicillin G, *New Engl. J. Med. 251*:466-71, 1954.
24. Massell, B. F., Sturgis, G. P., Knobloch, J. R., Streeper, R. B., Hall, T. N. and Norcross, P. Prevention of rheumatic fever by prompt penicillin therapy of hemolytic streptococcic respiratory infection, *J.A.M.A. 146*:1469-74, 1951.
25. Denny, F. W., Wannamaker, L. W., Brink, W. R., Rammelkamp, C. H., Jr. and Custer, E. A. Prevention of rheumatic fever, treatment of the preceding streptococcic infection, *J. Amer. med. Assoc. 143*:151-53, 1950.
26. Wannamaker, L. W. Rammelkamp, C. H., Jr., Denny, F. W., Brink, W. P., Houser, H. B., Hahn, E. O. and Dingle, J. W. Prophylaxis of acute rheumatic fever by treatment of the preceding streptococcal infection with various amounts of depot penicillin, *Amer. J. Med. 10*:673-96, 1951.
27. Stollerman, G. H. Prevention of rheumatic fever, *Amer. Practit. Dig. Treat. 5*:589-92, 1954.
28. Eagle, H., Fleishman, R. and Musselman, A. D. Effect of schedule of administration on the therapeutic efficiency of penicillin, *Amer. J. Med. 9*:280-99, 1950.
29. Houser, H. B. and Eckhardt, G. C. Recent developments in prevention of rheumatic fever, *Ann. intern. Med. 37*: 1035-43, 1952.
30. Morris, A. J., Catanzaro, F. J., Chamovitz, R., Stetson, C. A. and Rammelkamp, C. H. The relative importance of antigen and antibody in the pathogenesis of rheumatic fever (abstract), *Clin. Res. Proc. 2*:75, 1954.
31. Breese, B. B. and Disney, F. A. The accuracy of diagnosis of beta streptococcal infections on clinical grounds, *J. Pediat. 44*:670-73, 1954.
32. Rammelkamp, C. H., Jr., Houser, H. B., Hahn, E. O., Wannamaker, L. W., Denny, F. W. and Eckhardt, G. C. The prevention of rheumatic fever, in *Rheumatic fever, a Symposium*, edited by L. Thomas, Minneapolis, Univ. of Minn. Press, 1952, pp. 304-315.

THE CURRENT STATUS OF THE DEVELOPMENT OF ANTIMICROBIAL AGENTS*

GLADYS L. HOBBY, Ph.D.

Research Division, Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

IT is a privilege indeed for me to have been invited to discuss this evening the current status of the development of antimicrobial agents. To consider how far one has advanced in any field of endeavor is invariably worthwhile, for it forces one to give consideration also to that which has not been accomplished.

Much of what I shall say tonight will be historical in nature, for, as pointed out recently by a well-known scientist:

> "The past underlies the present, qualifies and moderates it, in some ways limits it and in some ways enriches it. . . .
>
> "It is a recurrent experience of scientific progress that what was yesterday an object of study, of interest in its own right, becomes today something to be taken for granted, something understood and reliable, something known and familiar – a tool for further research and discovery."[1]

* * * *

Like many developments in science, knowledge of the antimicrobial agents has grown in haphazard fashion. Isoniazid (isonicotinic acid hydrazide), one of the most powerful antimicrobial agents in use today, was first synthesized by Meyer and Mally[2] in 1912. By that time, sulfanilamide already had been synthesized by Gelmo in 1908.[3] Paul Ehrlich already had called attention to the potentialities of chemotherapeutic agents, and microbiologic techniques for their study already had been well established by Pasteur, Koch, and others. It was not until a quarter of a century later, however, that attention was focused specifically on evaluation of the potential significance of sulfanilamide, its

* Presented at the 27th Graduate Fortnight of The New York Academy of Medicine, October 25, 1954.

derivatives, and other similarly active compounds. It was not until forty years later that the chemotherapeutic properties of isoniazid were demonstrated.

Two decades have now elapsed since the chemotherapeutic activity of Prontosil rubrum was first described by Domagk[4] and since Levaditi and his associates[5] first demonstrated that the para-aminobenzene sulfonamide group breaks away from the rest of the Prontosil molecule in vivo, and is responsible for its chemotherapeutic properties. The subsequent synthesis of an extensive series of related compounds and definition of the scope and limitations of these agents, coupled with the timely observations of Dubos[6] on the action of gramicidin, served as a stimulus to Florey and his co-workers[7, 8] in their studies of the action of penicillin. Together, the observations made on these compounds introduced chemotherapy on a sound basis into medical practice.

The results obtained with the sulfonamides unquestionably were dramatic in nature. The remarkable effects achieved with penicillin, however, and the announcement of the discovery of streptomycin within a few years after its introduction, placed emphasis upon the prevalence of antibiotic-producing microorganisms in nature. Interest in biologic sources of these compounds was in no way diminished by the early difficulties encountered in the production of penicillin on a large-scale basis. In the search for additional compounds with chemotherapeutic properties, attention was thus focused for almost ten years thereafter upon those species of microorganisms which could be cultivated in quantity. Efforts in this direction were rewarded by the discovery of the tetracycline group of antimicrobials and numerous other compounds with specific although more limited action. It was perhaps the success of the carefully designed large-scale microbiologic studies, conducted during these years, that drew attention away from the field of synthetic chemicals. It is the success of these studies, perhaps, that accounts for our being largely dependent, in the year 1954, upon chemotherapeutic agents produced by organisms occurring in nature, although fully aware of the potentialities of synthetic chemicals.

One may justly wonder, at this time, if progress in the field of chemotherapy perhaps has been retarded by our having placed so much emphasis on those agents produced by living microorganisms. If as much emphasis had been placed, during the past ten years, upon evaluation of the chemotherapeutic properties of known or easily synthesized

chemical compounds, would greater strides have been made toward the control and eradication of infectious disease? Will the presently accepted and highly effective antibiotic agents be replaced in the future by synthetically-derived chemicals, as quinine has been replaced by chloraquin and primaquin?

It is of interest, perhaps, that, in the year 1953, the production, within the United States, of antibiotics alone totalled 2.1 million pounds, while the total sales exceeded 1.85 million pounds. According to a recent preliminary report of the United States Tariff Commission,[9] this represents a sales value of over 251 million dollars. According to this same report, an additional 4.7 million pounds of sulfa drugs, with a sales value of nearly 17 million dollars, likewise were produced during this same year. These figures do not include isoniazid, para-aminosalicylic acid, the sulfones, or other such synthetically-produced compounds. If these had been included, the total sales value of specific antimicrobial agents, within the United States alone, might well have approached 300 million dollars. By way of comparison total medicinal sales within the United States during 1953 amounted to 54.2 million pounds, with a total dollar value of 409.1 million.

The fact that such quantities of the antimicrobial agents were produced and marketed within a single year is indicative of the position which they have assumed in the health and economy of our nation. The ready acceptance of such tremendous amounts is in a sense a measure of their established usefulness.

Production figures are not available for most countries other than the United States. It is known, however, that the production of antibiotics has spread to the four corners of the world, and, while the United States is still the leading manufacturer of these products, they are now being fermented on all five continents. In the Western Hemisphere, private firms are fermenting penicillin in Brazil, Argentina and Canada. In Europe, the United Kingdom is producing about double its home consumption and exporting the remainder all over the world. Indeed, it has been reported that 97,360 billion international units of penicillin, i.e., equivalent to 128,670 pounds, were produced in Great Britain during 1953.[10] The German pharmaceutical industry has shared in the rapid recovery of the whole German economy and it now produces penicillin and streptomycin for export as well as for its local needs. Both Italian and French pharmaceutical companies, furthermore,

have taken up antibiotic fermentation on a large scale, while the Scandinavian countries are represented in the picture with Sweden and Denmark also producing penicillin. In the Far East, Japan is becoming a major exporter of antibiotics, while penicillin fermentation is being undertaken not only by private companies but also by government laboratories in countries such as India and Australia. Indeed, in the latter country, chloramphenicol likewise is produced currently. Behind the "iron curtain," there are five plants about which we have received news – two in Poland, one in Hungary, one in Rumania, and another in East Germany which are producing chloramphenicol.

In addition to those antibiotics actually produced in foreign countries, however, exports of antibiotics from the United States to other countries totalled, in 1953, more than 310,000 pounds, with a sales value of over 90 million dollars.[11] They reached during the past year essentially all countries except those within the "iron curtain." It is unfortunate, however, that, despite the efforts of the World Health Organization and other similar agencies, economic conditions do not permit the antimicrobials to exert their full effectiveness in those very areas where the need for them is greatest. While economic conditions in many countries undoubtedly are influenced in large part by the health of the people of that nation, local taxes make the antimicrobial drugs prohibitive, generally increasing the cost to double that in the United States. Governmental regulations, local beliefs and customs, and at times superstition, furthermore, restrict their usage.

The development and large-scale production of antimicrobial agents may be considered today to be "an art," – a skill dependent upon practice and performance and acquired by experience, study and observation. Insofar as the antibiotics are concerned, increased knowledge concerning strain mutations has contributed significantly to their availability. The application of engineering principles to the design, developmental and operating phases of fermentation processes has accounted, however, in far greater measure for the phenomenal increase in their output.

Penicillin, streptomycin and dihydrostreptomycin, tetracycline, oxytetracycline, and chlortetracycline, chloramphenicol, the sulfonamides, isoniazid, and para-aminosalicylic acid are among the most widely used antimicrobials today. In addition, neomycin, polymyxin B, bacitracin, erythromycin and carbomycin, viomycin, tyrothricin, stil-

bamidine, and the sulfones are used in special circumstances. Mention should be made also of pyrazinamide for, although not widely available at present, frequent reference will be made to it in the present discussion.

The sulfonamides, isoniazid, para-aminosalicylic acid, the sulfones, stilbamidine, and pyrazinamide are produced by chemical synthesis only. The chemical structures of penicillin, streptomycin, chloramphenicol, and the three tetracyclines furthermore have been described, while chloramphenicol, originally produced through a fermentation process, has now been synthesized chemically and indeed is produced commercially by a synthetic process.

The antimicrobial agents which are in use today are essentially ones that "happened to be effective"; they came into clinical use through the process of "survival of the fittest." No information was available originally to explain why these, and not others, were effective. Despite the fact that the element of chance strongly influenced both their discovery and the development of our knowledge concerning them, however, certain principles have been established, and certain concepts introduced. Today, we find ourselves attempting to evaluate these principles and concepts, and attempting, in a very human and totally inadequate manner, to introduce rationality into a problem that perhaps will always defy rationalization.

* * * *

It has been recognized for some time that the two basic principles of antimicrobial therapy undoubtedly are: 1) *To be effective, an antimicrobial agent must be capable of exerting its growth inhibiting action in vivo against the specific microorganism concerned;* and 2) *once within the body, it must reach the site at which the microorganism resides in sufficient concentration and must remain there for a sufficient period of time to permit it to exert its effects.* Many factors, however, operate to prevent a drug from acting in vivo, and evaluation of those hurdles which a good chemotherapeutic agent must surmount in order to survive is essential before a rational approach to chemotherapy can be introduced.

Of prime importance is the fact that susceptibility of a given microorganism to a given antimicrobial agent in vivo is a necessary prerequisite for chemotherapeutic effectiveness. Organisms resistant to a given agent

in vitro are unlikely to be susceptible in vivo unless, as was the case with Prontosil rubrum, that compound is converted within the host to another substance capable of inhibiting growth of the microorganism in question. A compound exhibiting a significant degree of antimicrobial activity in vitro, on the other hand, may or may not show similar activity within the host. The degree to which in vitro and in vivo effects correspond varies with the compound in question, and is dependent in large part upon its pharmacologic properties. Susceptibility of an organism to a given agent is an essential prerequisite; it is not an adequate prerequisite for drug effectiveness.

The notion that bactericidal action in vivo may be essential to fully efficient antimicrobial therapy is currently receiving widespread consideration. It is difficult, of course, to estimate the degree of bacteriostatic or bactericidal action that may result within the host. Furthermore, one cannot state with certainty the extent to which an antimicrobial agent may be responsible for the effect observed, or the extent to which the resistance of the host may influence the end result.

In those instances in which control of an acute infection by an antimicrobial agent has implied to the casual observer that the invading microorganisms have been eradicated by that antimicrobial, actual evidence to this effect has not been forthcoming. In this regard it is of interest, perhaps, that extensive study of morphologically healed tuberculous lesions, removed from patients who had received chemotherapy for periods ranging from four to twelve months, has indicated that tubercle bacilli remain viable in the majority of instances, and that the viability of these organisms can be demonstrated.[12] Undoubtedly not all of the bacilli present in such lesions are viable; nonetheless, a portion is alive. They can be cultivated in vitro, and presumably they could multiply in vivo and bring about relapse under appropriate conditions. The evidence with respect to this particular infection emphasizes the fact that it is seldom, if ever, possible to eradicate every single member of a particular infecting microbial population.

The very fact that chemotherapy merely slows or halts multiplication of the microorganism implies that the success of chemotherapy must depend upon the ability of the patient to control the infection after the administration of the drug has been discontinued. Chemotherapy may speed the process of healing, or may increase the frequency of success in an individual; it cannot alter the basic mechanism of healing.

Various factors which conceivably may act to prevent sterilization of lesions within the host have been discussed recently by McDermott,[13] who has offered three possible explanations for the phenomenon. It is of interest to consider these in detail, for in so doing attention is focused upon those notions and concepts which reflect the extent of present knowledge concerning the action of antimicrobial agents in vivo. Many of the points which will be mentioned may well have been discussed previously during this Fortnight. They will be mentioned again at this time, however, in order to show how they relate to each other and perhaps contribute to the gradual development of a rational understanding of chemotherapeutic action.

• • • •

Failure to sterilize lesions may be due to the fact that:

1. *Certain of the parasites are so situated within the body that they are not reached by the antimicrobial drug:* As stated previously, abundant evidence is available to indicate that an antimicrobial agent will be effective in vivo only if it reaches the site of the invading microorganism in a concentration sufficient to inhibit its growth. An understanding of the pharmacologic action of a drug, therefore, is essential in evaluation of its degree of chemotherapeutic activity and in evaluation of the manner in which it can most effectively be administered.

Experience with penicillin, more than with any other agent, has served to emphasize the variable pharmacologic effects that may be obtained with different forms of a single substance, and the extent to which distribution of a drug within the body may be influenced by alterations in the molecule, or by use of appropriate carrying vehicles. For example, it is well known that the rapid absorption and excretion of sodium or potassium penicillin G when administered in aqueous solution, although at times advantageous in that peak concentrations are reached in the blood within a few minutes, makes administration of the drug difficult in many instances. This rapid absorption is due in large part to the high solubility rate of these particular penicillin salts. Procaine penicillin, in contrast, is a salt which is much less soluble in water, and long-sustained concentrations of penicillin are obtained in the blood and urine following administration of this form of the antibiotic. In like manner, the sparingly soluble salt, benzathine (N, N^1-dibenzyl ethylenediamine dipenicillin), is released slowly from tissue

depots, with resultant prolonged levels of penicillin in the blood following its administration. If a substance is rapidly absorbed, the normal process of excretion may reduce its effectiveness. Conversion to a less soluble form, however, may increase its chemotherapeutic potentialities, while control of crystal or particle size may further regulate the rate of solubility and thus the speed with which it is released into the general circulation. Through an understanding of the chemical and physical properties of a compound one may enhance significantly its potentialities.

To date, *only* those antimicrobial agents capable of distributing themselves throughout the extracellular fluids of the body and capable of exerting demonstrable activity within such fluids have come into widespread use. In general, these compounds have been most effective in the control of acute microbial infections in which the invading microorganisms have been located in readily accessible areas within the host. In such instances, in vivo susceptibility of the invading microorganism to an antimicrobial agent has generally been a sufficient basis for the assumption that a therapeutic response would be achieved with that antimicrobial, provided adequate dosage was used. Distribution of the drug throughout the extracellular fluids of the body with measurable concentrations in the blood presumably has created in such persons a situation in which the organisms were as vulnerable to attack by the antimicrobial as they would have been in an in vitro system.

The two cardinal principles of antimicrobial action, namely in vivo susceptibility of the invading microorganism to the agent concerned, and distribution of adequate quantities of that agent to the site at which the organism resides, imply that in some situations distribution of the drug throughout the extracellular fluids of the body may not be sufficient.

In chronic types of infection, the invading microorganisms generally are located in areas of necrosis. The ability of streptomycin and isoniazid to penetrate into necrotic lesions has been demonstrated by microbiologic assays on resected tissue, but no such data are available with respect to other antimicrobial substances. Unquestionably, an antimicrobial will be fully effective in the control of suppurative lesions only if capable of penetrating into necrotic tissue; and, what is probably of greater importance, it will be fully effective against such lesions only if capable of exerting full antimicrobial activity in the presence of necrotic tissue. Since many bacilli often exist within monocytes, it is apparent, furthermore, that an antimicrobial agent will be fully effec-

tive in the control of such infections only if capable of traversing the cell boundary. Indeed, it is not unreasonable to assume from the data presented by Mackaness[14] with respect to antituberculous agents that the relative activities of antimicrobial agents in the control of those infections in which microbial cells may exist within monocytes may be related in very large part to the ease with which they traverse cell boundaries and to the degree of activity which they can exert in an intracellular environment.

2. *That certain members of the microbial population are subsisting at such a low level of metabolic activity throughout the entire period of chemotherapy that they are physiologically insusceptible to the action of the drug:* It is unfortunate indeed that relatively little is known concerning the exact mechanisms by which antimicrobial agents exert their effects against individual microorganisms. Knowledge concerning the biochemical and enzymatic processes responsible for bacterial growth and reproduction has increased rapidly in recent years, and numerous studies have been conducted to determine the effect of antimicrobial agents upon these processes.[15] Of particular significance in this respect are the recent studies by Wisseman and his associates[16] which indicate that the introduction of chloramphenicol into a rapidly growing culture of Escherichia coli is followed by abrupt cessation of protein synthesis, although ribonucleic acid and desoxyribonucleic acid continue to be synthesized at an unaltered rate after protein synthesis has ceased. As pointed out by Wyss,[15] however, and as shown so clearly by the studies of Wisseman and his associates, when a cell stops growing, some cell reactions continue, but many of them stop; the problem is to determine which stoppages are causal and which are resultant of the growth inhibition. Unquestionably, however, the vast majority of antimicrobial agents studied to date are most active against rapidly metabolizing cells, and conditions which slow the metabolic rate may simultaneously diminish antimicrobial activity.

Dubos,[17] in a recently published book discussing the "Biochemical Determinants of Microbial Diseases," has summarized various factors within necrotic tissue that conceivably may suppress the metabolic activities of at least certain species of microorganisms and thus prevent an antimicrobial agent from acting against them. Low oxygen tension, local acidity, the presence of free fatty acids and other antimicrobial agents liberated by autolytic processes during necrosis, as well as other

factors, contribute to the development of an environment capable only of suppressing metabolic processes.

McDermott and Tompsett[18] recently reported that pyrazinamide and nicotinamide are highly active tuberculostatic agents when tested at the pH of inflammatory tissue, e.g., pH 5.0 to 5.5, but that these compounds show virtually no activity when used within the so-called physiologic ranges of pH. According to these investigators, the pyrazinamide and nicotinamide activation in the acidic environment, with no demonstrable activity at neutrality, is a distinctly unusual phenomenon among the antibacterial drugs which have been in general use. None of the others tested, with the possible exception of penicillin,[16] exhibits its maximal activity when the environment becomes more acid.

In 1944, at the Seventeenth Graduate Fortnight held at this Academy, Dubos,[19] in a discussion of the mode of action of chemotherapeutic agents in vitro, commented:

> "Another factor worth considering in analyzing reasons why so many antibacterial agents are ineffective on infected wounds or burns is that the reaction of these tissues is often more acidic than that of the blood. It is a known fact, on the other hand, that most antibacterial agents become less active as the pH is lowered. One may wonder, therefore, whether the problem of infected wounds and burns will not demand that attention be directed toward a class of substances exhibiting an optimum zone of activity slightly on the acid side."

Only now is this important fact receiving consideration. As stated by McDermott and Tompsett,[18] however:

> ". . . from what little is known about the biochemical environments of infectious processes, it is clear that they are quite complicated and probably subject to rapid changes in character. Consequently, although the biochemical environment of the lesion is probably responsible for most chemotherapeutic failures, it is unlikely that any one environmental factor, such as the degree of acidity, will represent the sole determinant of the antimicrobial activity of a drug."

The evidence unquestionably is inadequate. Insufficient is known concerning the mechanism by which antimicrobial agents act specifically to inhibit growth of microbial cells; insufficient is known concerning the factors within the host that may interfere with such inhibi-

tory effects. Nonetheless, based on that evidence which does exist, it seems highly unlikely that any of the currently available antimicrobial agents, except possibly pyrazinamide, – even if capable of penetrating into necrotic tissue, – would be fully active against microorganisms located therein. This remains one of the major unsolved problems in the field of antimicrobials today, and imposes an important limitation upon their scope.

3. *That the microorganisms which survive represent the more drug-resistant fraction of the total infecting population:* In 1944, Bigger[20] reported that penicillin may fail to sterilize large populations of staphylococci in vitro because of the presence of so-called "persisters," i.e., organisms which are not undergoing cellular division and against which penicillin, therefore, is capable of exerting little or no effect. It was suggested that, in some instances, failure to cure staphylococcal infections in man with penicillin might be due to the presence of similar "persisters" in vivo. Such cells were estimated to be few in number, rarely exceeding one per million bacteria.

Other investigators[21, 22] have similarly observed small numbers of organisms, within otherwise susceptible strains, *not* inhibited by penicillin and yet with penicillin-susceptible progeny.

The difference between the so-called "persisters" and resistant cells is, in part, that the former, on subculture, give rise to drug-susceptible progeny, while the latter give rise to resistant cells; it is in part a quantitative difference. Whereas the former exist in small numbers and merely prevent total sterilization, the latter readily increase in number and interfere also with the degree of bacteriostatic effect observed.[23] The eradication, therefore, of "persisters" presents a totally separate problem than does the control of drug-resistant strains.

It is scarcely necessary to comment on the emergence of drug-resistant strains. It is well known that microbial strains may be susceptible to one or more antimicrobial agents, they may be naturally resistant to such agents, or they may be resistant due to previous contact with the antimicrobial. Unquestionably, resistance develops more rapidly to some agents than to others, but perhaps of greater importance, is the fact that emergence of drug resistance is more frequent among some species of microorganisms than others. Indeed, what evidence there is at present suggests that quite possibly resistance becomes a problem only when dealing 1) with those microorganisms capable of adapting themselves

readily to growth under various or adverse circumstances, or 2) with those species which are difficult to identify by serological or biochemical means and perhaps may be considered to be "impure" in the sense that the population within a single strain conceivably may be comprised of representatives of various strains. Emergence of resistance has rarely, if ever, been noted among those microbial species with the most fastidious growth requirements, e.g., Streptococcus hemolyticus, Diplococcus pneumoniae, etc.

It is of interest in this regard to recall, perhaps, that each discovery in medicine uncovers a problem. Just as the introduction of the sulfonamides and penicillin uncovered the disease, primary atypical pneumonia, so also the widespread use of streptomycin, isoniazid, and para-aminosalicylic acid has led to recognition of the possible pathogenic significance of certain "atypical acid-fast bacilli" while the widespread use of the so-called "broad-spectrum antimicrobial agents" has aroused speculation concerning the nature and pathogenicity of staphylococci and certain other microbial species.

The appearance of resistant strains in vivo has been associated in large part with changes in microbial flora resulting within the host from administration of the antimicrobial agents. The occurrence of similar changes in flora, when species of microorganisms present in a given area are eliminated, is a natural phenomenon in nature. The new population, present after antimicrobial therapy, does not necessarily consist of drug-resistant variants of the organisms originally present in the individual patient's flora; rather, it consists of organisms which presumably were previously incapable of competing against those normally present within the host but now are able to grow and multiply in the absence of such competing cells.

It is reasonable to assume that occasionally a newly established organism may possess some degree of pathogenicity. *Only in rare instances*, however, have such microorganisms, — newly introduced into a host and/or increasing to predominant numbers within the host as a result of antimicrobial therapy, appeared to be pathogenic. In these few instances, surgical intervention generally has been associated with systemic invasion by the microorganisms, and it is not unlikely that factors associated with trauma may be essential to entry of these organisms into the body and to subsequent establishment of systemic infection. The nature of these factors has not been determined. It is of interest

in this regard, however, that Hammond and her associates[24] have described a strain of Pseudomonas aeruginosa, which on oral inoculation is quite harmless for mice, but which is rapidly fatal in irradiated mice infected on the eleventh day post irradiation. Since susceptibility to the infection is less on the fifth day post irradiation, and least of all directly after irradiation, and since damage to the intestinal mucosa is maximal within the first few hours after irradiation, these investigators conclude that the increased susceptibility of these animals is *not* due to increased permeability of the mucosa of the gut, but to impairment of the animal's natural defenses against infection.

Diverse opinions exist as to the long-range significance of the emergence of microbial strains resistant to the presently available antimicrobial agents. Nonetheless, as recently pointed out by Dowling,[25] a resistant strain, although of considerable importance to the individual harboring it, undoubtedly must be transmitted to other susceptible hosts if it is to assume larger significance.

> "The number of resistant bacteria in any given community will be directly proportional to the number of carriers of resistant bacteria, the number of susceptible hosts, and the frequency and intimacy of contact between the two. The spread of resistant strains may be prevented by the use of proper isolation technics or by raising the resistance of the host to the microorganism concerned."

One might add that the spread of resistant strains may be prevented also by use of antimicrobial agents only when there is a specific indication for such therapy, and then only in full therapeutic dosages. The currently prevalent trend to administer these drugs for prophylactic purposes is one which may well lead to unfortunate circumstances in the future.

* * * *

In summary, therefore, we find that the past ten to fifteen years have seen the introduction of large numbers of antimicrobial agents capable of controlling infectious diseases produced by a wide variety of pathogenic microorganisms. These ten to fifteen years have seen the development of methods for producing these agents within the United States in quantities sufficient to treat well over 100 million patients yearly, while the manufacture and distribution of these agents has been

extended throughout the world. During these ten to fifteen years, the mechanisms by which these agents exert their effects, both in vitro and in vivo, have been partially elucidated, and the conditions under which they may fail to act to some extent recognized.

The events of the past decade have brought us to a point at which one can no longer deny or question the value of these compounds as chemotherapeutic agents, when used either singly or in combination in appropriate situations. Yet they have brought us also to a point at which it is recognized that the limits of chemotherapy unquestionably are set by the amount of tissue destruction that has taken place before drug therapy is started, and by the sterilizing capacity of the drug or combination of drugs used. It is now a well accepted fact that the limits of chemotherapy can be extended only by the defense mechanisms of the body and/or by surgical intervention. It is with this in mind that Smadel, Woodward and their associates[26] are investigating the relation of the immune response to antimicrobial effects, while resectional surgery is widely used today in the treatment of tuberculosis. Quite probably it is for this reason also that the future will lie in attempts, already being made at least on an experimental basis, to alter the nature of the lesion by means of hormones, enzymes, or other such substances.

No mention has been made concerning the growth stimulatory properties of the antimicrobial agents. Of considerable economic importance, however, is the fact that minute amounts of penicillin, chlortetracycline, oxytetracycline, and bacitracin are capable of stimulating growth rates in poultry, while oxytetracycline and chlortetracycline are effective in swine. Many of the antimicrobial agents are capable likewise of stimulating growth rates of plants, and attempts are being made currently to elucidate the manner in which these effects are brought about.

Time does not permit comment concerning the various other uses being developed for the antimicrobial agents. The wide variety of conditions under which they may exert their growth inhibitory or growth stimulatory properties, however, continues to encourage the search for new ones.

In the development of new antimicrobial agents, the element of chance, once so important in antibiotic research, still influences the initial isolation of a strain capable of producing an antimicrobial sub-

stance; it influences the selection of the proper group of chemical compounds for study and evaluation. Techniques for the rapid testing of the antibiotic-producing properties of microorganisms, and knowledge concerning the growth requirements and optimum environmental conditions for growth of these organisms, however, has facilitated the search for new compounds. Paper chromatography, a technique designed to separate minute quantities on specially treated filter paper strips, allows rapid detection of new compounds or different forms of the same substance. By this technique, the identities of compounds may be established and their activities indicated even before isolation and purification are possible. Refined methods similarly facilitate the recovery and purification of those compounds warranting production in quantity, while infra red, ultra violet absorption, and Craig counter current distribution techniques aid in the analysis of the fractions isolated.

Experience with penicillin, streptomycin, the tetracyclines, and the other antimicrobial agents has established techniques for evaluation of new ones, and baselines for comparison. Despite this, however, speculation strongly influences one's interpretation of the potentialities of a compound, as suggested by experimental observations. The problem today is to choose among the many active compounds isolated. Not all can be produced on a large scale, for there is competition for fermentation tanks, for equipment for their recovery or synthesis, for animals and indeed suitable patients for their evaluation. One must select those that will be studied extensively and the very notions and concepts which we have discussed tonight form the basis on which the decision must be reached today. Without doubt, these notions at times may lead to erroneous decisions, and the element of chance is eliminated only insofar as our understanding of the mechanism by which these agents act in vivo may be correct.

In evaluating a new antimicrobial agent today, it is not enough to know that it is capable of exerting chemotherapeutic effects. Instead, one must ask: How effective is it in comparison with other available agents? Can it act against microorganisms located within necrotic tissue, as well as in the blood and extracellular fluids? Is it fully effective against microorganisms located intracellularly? Is it less likely to cause side reactions than other compounds covering the same disease spectrum? By what route must it be administered, and what will be its cost to the patient per day? If not suitable for systemic administration to

humans, does it have potentialities as a topical agent, or does it have industrial or agricultural uses? If not powerful enough to act alone, can it act synergistically?

The problem today is no longer a simple one. Furthermore, it is no longer centered merely on increasing the number of antimicrobial agents available. It is centered rather on "tailor-making" them to fit a specific need. Just as demonstration of the chemotherapeutic action of the sulfonamides stimulated interest in the action of penicillin, so also the discovery of many antimicrobial agents in nature has stimulated the desire to synthesize them chemically and to seek out those biochemical and enzymatic processes through which they exert their effects.

Unquestionably time and experience will clarify our understanding of these compounds and of the manner in which they act, either in an in vitro environment or within the host, to diminish or to stimulate growth rates. At present, one can only speculate.

REFERENCES

1. Oppenheimer, J. R. *Science and the common understanding.* New York, Simon and Schuster, 1953.
2. Meyer, H. and Mally J. Über hydrazinderivate der Pyridincarbonsauren, *Monatsh. Chem. 33*:393-414, 1912.
3. Long, P. H. and Bliss, E. A. *The clinical and experimental use of sulfanilamide, sulfapyridine and allied compounds.* New York, Macmillan Co., 1939.
4. Domagk, G. Ein Beitrag zur Chemotherapie der bakteriellen Infektionen, *Deutsch. med. Wschr. 61*:250-53, 1935.
5. Levaditi, C., and Vaisman, A. Action curative du 4'-sulfamido-2', 4'-diaminonzobenzène et de quelques dérivés similaires dans le streptococcie experimentale, *C. R. Soc. Biol. 119*:946-49, 1935.
6. Dubos, R. J. Studies on a bactericidal agent from a soil bacillus: the protective effect of this bactericidal agent against experimental pneumococcus infections in mice, *J. exp. Med. 70*:11-17, 1939.
7. Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J. and Sanders, A. G. Penicillin as a chemotherapeutic agent, *Lancet 2*:226-28, 1940.
8. Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. S. and Florey, H. W. Further observations on penicillin, *Lancet 2*:177-88, 1941.
9. U.S. Report No. 194, 2nd Series (GPO-CL. No./TC 1.9:194), *U.S. Tariff Commission preliminary report on synthetic organic chemicals, U.S. production and sales, for the year 1953.*
10. *Monthly digest of statistics,* published by the Central Statistical Office, London, August 1954, p. 134, table 160.
11. U.S. Report No. FT410, *U.S. exports of domestic and foreign merchandise commodities by country of destination,* published by the U.S. Department of Commerce, Bureau of the Census, part II, 1953, pp. 160-161.
12. Hobby, G. L., Auerbach, O., Lenert, T. F., Small, M. J. and Comer, J. V. The late emergence of M. tuberculosis in liquid cultures of pulmonary lesions resected from humans, *Amer. Rev. Tuberc. 70*:191-218, 1954.
13. McDermott, W. Host factors in chemotherapy, in *Bacterial and mycotic infections of man,* edited by R. J. Dubos, 2.ed., Philadelphia, J. B. Lippincott Co.,

1952, pp. 744-765.

14. Mackaness, G. B. and Smith, N. The bactericidal action of isoniazid, streptomycin and Terramycin on extracellular and intracellular tubercle bacilli, *Amer. Rev. Tubere. 67*:322-40, 1953.
15. Wyss, O. Introduction to the symposium on the mode of action of antibiotics, *Bact. Rev. 17*:17-19, 1953.
16. Wisseman, C. L., Jr., Smadel, J. E., Hahn, F. E. and Hopps, H. E. Mode of action of chloramphenicol; action of chloramphenicol on assimilation of ammonia and on synthesis of proteins and nucleic acids in Escherichia coli, *J. Bact. 67*:662-73, 1954.
17. Dubos, R. J. *Biochemical determinants of microbial diseases.* Cambridge, Mass., Harvard University Press, 1954. (Harvard University monographs in medicine and public health, no. 13.)
18. McDermott, W. and Tompsett, R. Activation of pyrazinamide and nicotinamide in acidic environments in vitro, *Amer. Rev. Tubere. 70*:748-54, 1954.
19. Dubos, R. J. The mode of action of chemotherapeutic agents, *Bull. N. Y. Acad. Med. 21*:27-36, 1945.
20. Bigger, J. W. The bactericidal action of penicillin on Staphylococcus pyogenes, *Irish J. med. Sci.* 1944: 553; 585.
21. Hobby, G. L., Meyer, K. and Chaffee, E. The activity of penicillin in vitro, *Proc. Soc. exp. Biol. Med. 50*:277-80, 1942.
22. Tompsett, R. Unpublished observations, 1954.
23. Eagle, H. Experimental approach to the problem of treatment failure with penicillin; group A streptococcal infection in mice, *Amer. J. Med. 13*:389-99, 1952.
24. Hammond, C. W., Colling, M., Cooper, D. B. and Miller, C. P. Studies on susceptibility to infection following ionizing radiation; its estimation by oral inoculation at different times post irradiation, *J. exp. Med. 99*:411-18, 1954.
25. Dowling, H. F. The effect of the emergence of resistant strains on the future of antibiotic therapy, in *Antibiotics annual, 1953-1954*, New York, N. Y., Medical Encyclopedia, Inc., 1953, pp. 27-34.
26. Woodward, T. E. Problems of immunity in non-tuberculous infections, *Trans. Conf. on Chemotherapy of Tuberculosis* (held under the auspices of the U.S. Veterans Administration, Army and Navy) *13*:255-57, 1954.

ALEXANDER COCHRAN BOWEN—HARLOW BROOKS SCHOLARSHIP

This Scholarship was established to provide for advanced study, preferably abroad, in a clinical subject in preparation for the practice of medicine or surgery in any of its branches.

The Committee announces that it will receive applications which must be submitted by *March 31, 1955* for the award of *one* scholarship in 1955 under the conditions hereinafter stated.

Candidates must be male interns or residents in Municipal or Voluntary hospitals in New York City.

The Committee stipulates that the study be pursued abroad, preferably in Great Britain or Continental Europe. The course of study, the auspices under which it is to be taken and the place or places where the study is to be pursued must receive the approval of the Committee.

Since a period of residence and study abroad will require increased financial assistance the Committee will award in 1955 *one* scholarship grant in the amount of $2,500.00.

Candidates may secure application forms by addressing

The Executive Secretary, Committee on Medical Education
New York Academy of Medicine, 2 East 103 Street, N.Y. 29, N.Y.

CURRENT CONCEPTS IN THE MANAGEMENT OF ARTERIOSCLEROSIS

*Transcription of a Panel Meeting on Therapeutics**

WILLIAM DOCK . . . Moderator

Professor of Medicine, State University of New York, College of Medicine at New York City; Attending Physician (University Division) Kings County Hospital

Panel Members

DAVID ADLERSBERG

Assistant Clinical Professor of Medicine, College of Physicians and Surgeons, Columbia University; Associate Attending Physician for Metabolic Diseases, The Mount Sinai Hospital

HOWARD A. EDER

Assistant Professor of Medicine, Cornell University Medical College; Assistant Attending Physician, The New York Hospital

FORREST E. KENDALL, Ph.D.

Associate Professor of Biochemistry, College of Physicians and Surgeons, Columbia University

CHARLES F. WILKINSON, JR.

Professor and Chairman, Department of Medicine, New York University Post-Graduate Medical School; Visiting Physician and Director, Fourth Medical (N.Y.U.) Division, Bellevue Hospital

* Presented at the Monthly Panel Meeting (Second Series), February 5, 1954 at The New York Academy of Medicine.

MODERATOR DOCK: We are talking about the management of a disease and yet there is really no agreement about whether there is any management. At the present time experts in the field of study of atherosclerosis in man and in animals are not in agreement as to whether this disease is in any way controllable, in other words, whether there is any management for atherosclerosis. On the panel we have men who are studying this problem with experimental techniques in animals and in men. What we want to do is to give you different points of view. Finally, we will ask each one of the members of the panel what answer he would give a young patient who had a recent myocardial infarction and who asked him, "Is there anything I can do to control the rate at which this disease progresses?"

We will begin by asking each one of these men to give us some general idea of his point of view about the situation. I shall begin by asking of Dr. Forrest Kendall because Dr. Kendall, more than any of the rest of us, has been interested in the experimental work in animals. It was from Anitshkow's work, now nearly forty years ago, that there developed evidence that perhaps atherosclerosis was an acquired disease for which there might be some effective management. *I will start by asking Dr. Kndall, who is at the Goldwater Memorial and on the faculty of Columbia University, what he thinks, as of this afternoon, about the possibility that atherosclerosis in man may be an acquired disease.*

DR. FORREST E. KENDALL: I am convinced at the present time that arteriosclerosis should be considered a disease of metabolic origin. I am inclined to believe that faulty metabolism of cholesterol is involved in some way. Of course, in our experimental work most of the emphasis has been upon cholesterol because we have been able to produce lesions very similar to the lesions seen in clinical arteriosclerosis by maintaining high serum cholesterol levels in dogs, rabbits and quite recently in monkeys. There has been considerable question as to whether the same conditions are required for the production of clinical arteriosclerosis in man. It has been impossible to show a close correlation between the serum cholesterol levels commonly seen in the American population and the degree and severity of arteriosclerosis observed. For instance, many years ago Sperry and Landy examined a series of Medical Examiner cases, most of which represented accidental deaths. They analyzed serum and tried to correlate serum cholesterol levels with the degree

of arteriosclerosis, both generalized and coronary, found in these patients upon examination. They were unable to show that there was any correlation between the serum cholesterol level and the severity of the disease. But there is another interesting feature about this report. They found that all but three of the 101 cases over the age of thirty had definite arteriosclerosis of the aorta. Their findings might possibly be taken to indicate that the range of serum cholesterol levels found in the average American population is sufficiently high to cause arteriosclerosis. The range of values in an American population may not be normal values. They may represent hypercholesterolemia. If we examine a population from areas of the world where arteriosclerosis is not a universal disease, where the incidence of both generalized and coronary arteriosclerosis is distinctly low, we find this freedom from arteriosclerosis associated with low serum cholesterol levels. Likewise if we examine a selected population whose serum cholesterol levels are markedly higher than is considered to be normal for our population we find a much earlier and more severe involvement. So I think in clinical arteriosclerosis we have pretty much the same relationship between the serum cholesterol levels and the incidence of the disease that we can demonstrate in experimental animals. I don't think serum levels alone tell the whole story. I think the recent work on the lipoprotein fractions from Gofman's laboratory, and particularly the work in the alpha and beta lipoproteins from Barr's laboratory create the suspicion that the *nature* of the cholesterol in the serum may play just as large a role in the disease as does the level.

Barr has recently pointed out that if we compare the distribution of the cholesterol between the alpha and beta lipoprotein fractions in animals that normally show a very small incidence of arteriosclerosis with the ratio between the alpha and beta lipoproteins in human beings, the difference is great. Animals free from arteriosclerosis transport most of the cholesterol in the alpha lipoprotein fraction. Human beings transport most of it in the beta. We have been able to show that when animals are fed diets which produce hypercholesterolemia we get a shift of the cholesterol from predominantly alpha to predominantly beta lipoproteins. This may be the key to the problem.

MODERATOR DOCK: Dr. Kendall, Dr. Eder is here and will talk about some of these problems in man.

DR. KENDALL: One thing that we should consider in this problem

is the fact that the serum cholesterol is not a level level and distribution is not a property of the blood alone. The blood provides a transport mechanism and the level of cholesterol (or of any component) in the serum is the result of a balance between the rate at which this substance is poured into the blood stream and the rate at which it is taken out. We and others have obtained quite a bit of information about how cholesterol enters the blood stream. We know practically nothing about how it is taken out. I suspect that the factor that determines the level is located in the peripheral tissues, that the final level is determined by the ability of the cells throughout the body, muscle cells in particular, to utilize and dispose of cholesterol presented to them. I suspect that the level in an individual or an animal is under the control of the hormone system of the body. I am theorizing now. I think that the most hopeful line of approach in a program aimed at the control of arteriosclerosis is through a more complete investigation of the role of hormones in controlling metabolic processes in the cells.

MODERATOR DOCK: *I think we had better ask Dr. Adlersberg, who has been interested in the genetic background for a long time, as has Dr. Wilkinson, whether the patients that have hypercholesterolemia show other evidences of endocrine disturbance, because this problem has been raised by Dr. Kendall's suggestion. If so, is there any evidence that we can control the disease in patients with hypercholesterolemia by any sort of hormonal therapy? Dr. Adlersberg!*

DR. DAVID ADLERSBERG: I would like to begin by stating briefly that I also believe that atherosclerosis is a disease. I am much impressed by the change that has occurred during the last ten or fifteen years in the thinking of the clinician concerning atherosclerosis. Whereas previously it was considered to be just a phenomenon associated with the process of aging and was associated with the idea that nothing could be done about it, most of us now seem to believe that atherosclerosis is a disease. It is more frequent in old age, but it kills many people at a young age too. On the one hand, one finds among people over seventy, a small percentage indeed who are free of atherosclerosis although their vascular systems may present evidence of aging of the arteries in the form of elongation, dilatation, etc. On the other hand, one may find young individuals with very extensive atherosclerosis who may be killed by this disease at the age of twenty to thirty and sometimes even earlier. Now if this is a disease, then we ought to concentrate on the study

of its etiology, pathogenesis, clinical manifestations and, possibly also in the future, on treatment and prevention. For the latter point, I must admit that, in my opinion, there is no definite scientific basis as yet.

We have been particularly interested in the familial occurrence of atherosclerosis and of the disorder of lipid metabolism which may be associated with it. Although I believe that cholesterol is important in the pathogenesis of atherosclerosis in man, as well as in the experimental animal, I do not believe that we should limit ourselves only to cholesterol, or as a matter of fact, to lipids only. It is possible that the metabolic defect goes deeper than that and involves, for instance, certain protein-carbohydrate aggregates, mucoproteins, and possibly other substances as well. There is some evidence for that at present. In these same families one is struck by the fact that typical manifestations of the fully developed disease are not frequent. In other words, you don't find many members of these families who have the syndrome, say of xanthelasma, coronary artery disease, corneal arcus, and xanthoma tendinosum or tuberosum. In a generation of siblings there may be one or two who will present the whole syndrome, one or two who may present only the skin manifestations of xanthomatosis and some who will only present the chemical metabolic error of hypercholesterolemia. We know that hypercholesterolemia is only one important facet of lipid metabolism disturbance. Profound disturbance of lipoproteins is associated with hypercholesterolemia. I am sure this aspect of the problem will be discussed later.

As far as hormonal changes in our patients are concerned, it is very difficult to make a statement on that. Dr. Hamilton is of the opinion that some of these hypercholesterolemic patients probably have changes in their sexual sphere. We have the impression that some of these patients have rather small families. However, not much more can be said on that. We know that some hormones very definitely have an effect on lipid metabolism. Probably the most important are the thyroid and the female sex hormone. We believe that the adrenal cortex plays a role but the real extent of this control cannot be stated at present.

MODERATOR DOCK: *You feel at present that there is no indication for thyroid hormone therapy, for example, in managing your patients with hypercholesterolemia? You don't try to raise the level of thyroid activity by feeding thyroid?*

DR. ADLERSBERG: We have used thyroid in a number of these patients

and employed certain tests to see if we could evaluate the function of the thyroid. Only a small minority of them showed evidence of effect with thyroid hormone, while the others showed no effect.

MODERATOR DOCK: *Dr. Wilkinson, you also have been interested in the genetic background of some of these patients and one of the things that struck you was the families that had hypercholesterolemia and had very little coronary disease. Will you speak about this phase of the program?*

DR. CHARLES F. WILKINSON, JR.: Yes. Our approach was a little different from that of Dr. Adlersberg in that we studied large families. These were contacted not just because the patient was in the hospital and had either hypercholesterolemia or heart disease. We studied members of these families from several days after birth until almost eighty years of age. When hypercholesterolemia was the only abnormality present we found that there was the same life expectancy in each group. This was true of those people who did not have xanthoma. All they had was a primary hypercholesterolemia or what we term the syndrome of familial hypercholesterolemia. This is the place to point out that there are several different types of hypercholesterolemia which can be distinguished one from another. There is a primary hypercholesterolemia, the genetics of which has been fairly well established and then there is secondary hypercholesterolemia which may be due to the familial hyperlipemia where the basic metabolic fault is the inability of the body to remove neutral fat from the blood stream. Myxedema can cause a hypercholesterolemia, as may the nephrotic syndrome, diabetes, and so on. So when we speak of hypercholesterolemia we must be careful to define what type of hypercholesterolemia we mean.

MODERATOR DOCK: One point that has been emphasized is the relation to obesity. *Was obesity a frequently occurring feature in these families?*

DR. WILKINSON: Obesity was not a feature of these families. They were an offshoot of the Amish Church, by and large foreign. The people ate well, but those who lived in cities were just one or two generations away from the farm, and all were relatively hardworking people. We had only four or five cases of obesity in almost 300 people.

MODERATOR DOCK: *So obesity wasn't a feature of the families and they had no high incidence of coronary disease although they had hypercholesterolemia?*

DR. WILKINSON: Not in the homozygous abnormality, nor in the

hypercholesterolemia associated with xanthoma tuberosum.

MODERATOR DOCK: *Coronary disease?*

DR. WILKINSON: The youngest case of angina reported in that series was a girl of nine years, autopsied last year at the age of fourteen. She had survived, I think, three coronary attacks and died with the fourth.

MODERATOR DOCK: *There is one other aspect of obesity that I would like to ask Dr. Eder to talk to us about. In Dr. Barr's discussions of atherosclerosis he indicated that he thought that obesity was an important factor. I wonder if in your studies of alpha and beta lipoprotein and cholesterol you found there was a relationship between obesity and a relatively high level of alpha and beta lipoproteins?*

DR. HOWARD A. EDER: I believe that Dr. Barr's conclusions on this point perhaps did not come directly from our data but from a review of the literature. Since we have made no such studies, no conclusions regarding that question are possible from our data.

MODERATOR DOCK: *So in this group there was no direct relationship to obesity?*

DR. EDER: In our group we attempted to make no correlation.

MODERATOR DOCK: In the literature it is often stated that obesity is an important factor in coronary disease and I regret to say that my own name is on one such paper. Beginning about ten years ago, with Dr. Yater's cases in the Army, going on to Levine, White and Gertler's Boston series and that of Spain in Westchester County, it has been shown that people dying of coronary disease before the age of thirty-five in the Army, or forty-five in the civilian population, show the same number of subjects who are obese, ideal weight or underweight, as people killed in accidents or selected because they had the same body build and worked and lived in the same community as a patient with early coronary disease. Statistically the American literature now presents complete disproof of the thesis that obesity contributes to early coronary disease. Three entirely different series of studies have been reported, but their findings are all the same.

DR. ADLERSBERG: I would like to say a word on that. Dr. Zak and I had a small series of patients who died of coronary artery disease under the age of forty-six. This was post mortem material with clinical observations available. While in the younger men of this group obesity apparently was not a factor, we had the impression that among the women of this age group obesity could have been a factor. There was

a difference between this group and the control group, the average age of which was sixty-two.

MODERATOR DOCK: *Dr. Wilkinson, you have some observations?*

DR. WILKINSON: I think the obesity question is not quite as definitely and well proven as Dr. Dock has indicated. In the first place in speaking of death by coronary occlusion, one must remember that a little bit of coronary atherosclerosis in a wrong place is bad and one may have a lot in the body and live to die at ninety and die from something else. We should decide whether we are speaking of people who died of a coronary, period, or try to correlate the degree of atherosclerosis with the other factors. I would like to urge again, that these two approaches are entirely different. Dr. Willens in his study at Bellevue came to a different conclusion. While he appears to be outnumbered at the present time, one could probably find almost as much data on one side of the fence as the other. He studied people who died in Bellevue Hospital, and whether they died from coronary occlusion was not the point. The point was that these people had died and he tried to estimate the amount of atherosclerosis present. He found there was a greater degree as well as a greater incidence of atherosclerosis in those who were obese. In a second paper of his he presented data indicating that people who had been obese and then died of a wasting illness, so that they lost their obesity, had less atherosclerosis than he would have expected on the basis of his first series. He felt that this indicated that the process was reversible if weight was lost. Now there is a hooker in that, for we should bear in mind that when people die of a wasting disease a lot of things happen to their hormonal systems. It is somewhat risky to say that the loss of obesity was the cause of the diminished atherosclerosis. It might just as well be said that the stress under which these people were dying had some effect on the body hormones and thus on atherosclerosis.

MODERATOR DOCK: *Dr. Eder.*

DR. EDER: There are two points I would like to make: The first is in regard to the point raised as to the use of coronary artery disease as an index of atherosclerosis. In practice this is necessary because there is really no other index that we can use.

Recently in the department of pathology at Cornell, Dr. Hood made some observations in which he attempted to correlate the extent of the amount of cholesterol in the aorta with the extent of coronary artery

lesions. It turned out that there was quite a good correlation.

Secondly, in relation to the question of obesity, I also feel, purely from a review of the literature, that perhaps the question is still open. The data of Dr. Willens are important. Also it is of interest, in examining Yater's series of young individuals with coronary artery disease, that the number of them who were considerably overweight was greater than in the control group. Also, there are other series, such as the one recently reported by Master, and of course life insurance statistics, that suggest that obesity cannot be completely ruled out at the present time.

MODERATOR DOCK: That cheers me up because I am on record as thinking that obesity is related to the early coronary deaths in the Army in a small series that we studied. Dr. Yater had much more material.

From the standpoint of management this is very important. If obesity is an important factor predisposing to atherosclerosis and coronary disease, the doctor would presumably recommend to some patients that they try to bring their weight down far below the ideal for their age or even below the ideal for persons of their body build at maturity. All studies indicate that our population gets steadily heavier as it gets older. This course is quite unphysiologic. Your bones atrophy and your muscles atrophy as you get old and exercise less. The tables showing what the average person weighs at any given age, simply indicate the rate at which oily dropsy develops in our population. If obesity were the main factor in coronary disease, patients should try to bring their weight below the ideal at the time of graduation from high school.

Dr. Eder, you have been working with one method of studying the fractions of cholesterol in relation to coronary disease and you have some observations on hormone therapy as it affects the beta lipoproteins. Will you let us know whether there are therapeutic conclusions to be drawn from your data?

DR. EDER: The plasma lipids have been studied by a variety of different techniques. Originally, because cholesterol was the lipid determined in chemical laboratories, lipids were studied exclusively by the determination of cholesterol. As methods for determination of phospholipid and neutral fats became available all of these have been studied. In recent years attention has been directed to the fact that lipids don't circulate in the plasma as lipid alone but rather in association with certain proteins as lipoproteins. Two large groups of lipid-protein complexes are present in the plasma. The first group has been called the alpha lipo-

proteins because on electrophoresis they migrate with the mobility of alpha globulins. The second are beta lipoproteins and I use the word "lipoproteins" because I think each group probably consists of a variety of slightly different proteins.

The alpha lipoproteins have a molecular weight of about 200,000 and contain considerably more protein than there is in the beta lipoprotein. Perhaps 50 or 60 per cent of the alpha protein molecule is protein whereas in the beta lipoprotein only about 25 per cent of the molecule is protein and the rest is lipid. In the alpha lipoprotein the proportion of cholesterol to phospholipid by weight is in the ratio of 0.5. In other words, there is twice as much phospholipid in alpha lipoprotein as in beta lipoprotein.

The beta lipoproteins are substances of high molecular weight, about 1,200,000, 1,300,000. These molecules consist of cholesterol and phospholipid in the proportions of about 1.3. Thus there is more cholesterol in the beta lipoprotein than there is phospholipid as compared to alpha, in which there is much more phospholipid.

This brings us then to a consideration of the various levels that have been measured in the plasma. As Dr. Kendall has mentioned, cholesterol levels are oftentimes elevated in atherosclerosis. In a series of patients the mean level of cholesterol will be higher than the mean level of a control population of the same age and sex. On the other hand, individual patients will have serum cholesterol levels that fall well within the normal range.

The second development of recent years has been the emphasis on the cholesterol-phospholipid ratio. Data were obtained showing that patients with atherosclerosis had ratios of cholesterol to phospholipid which were higher than in normal individuals. Since phospholipid is a very soluble substance it was postulated that perhaps phospholipid served to solubilize the lipids. When the amount of cholesterol in relation to the amount of phospholipid became great, then the lipids became less soluble. It was noted by Man and Peters, quite a few years ago, that the cholesterol-phospholipid ratio rose with increasing cholesterol concentrations. In diseases where the cholesterol is elevated, you find high cholesterol-phospholipid ratios. Our data indicated that this is due to the fact that when cholesterol is elevated there is a higher proportion of the beta lipoprotein, which has a cholesterol-phospholipid ratio of 1.2 as contrasted to alpha which has a cholesterol-phospholipid ratio of 0.5.

With accumulation of beta lipoprotein, cholesterol-phospholipid ratio in the plasma will rise. In normal young women about a third of the cholesterol is found in the alpha lipoprotein fraction. We have separated these by Cohn chemical fractions. They may be separated by a variety of different methods and I do not believe there is any great advantage of one method over the other. I think they tell you pretty much the same thing, whether done by chemical means, by zone electrophoresis, or by ultracentrifugation. In any case, in young females about a third of the cholesterol is in alpha lipoprotein, and two-thirds in beta. Young males have a slightly different distribution with about a quarter of the cholesterol in the alpha and three-quarters in the beta. In the older age groups the alpha lipoprotein tends to decrease while the beta increases. Also the difference between men and women disappears.

In diseases such as atherosclerosis the amount of cholesterol in the alpha lipoprotein is uniformly low. The amount in beta is usually increased although it may not be. In diseases that are related to atherosclerosis, such as nephrosis, familial hypercholesterolemia, the amount of cholesterol in alpha lipoprotein is also very low.

I should like to note that in liver disease there is a marked alteration of the lipoprotein pattern. In liver diseases where there is biliary obstruction, or, I believe also in the experimental production of hyperlipemia by the injection of surface active substances such as "Triton" or "Tween," lipoproteins are produced which have phospholipid-cholesterol ratios of 0.5 and consist largely of free cholesterol. They migrate by electrophoresis as beta lipoprotein, act in the ultracentrifuge as beta lipoproteins.

MODERATOR DOCK: *There is some importance to this in practice, because these diseases do not produce atherosclerosis.*

DR. EDER: Yes.

MODERATOR DOCK: *Here you have high beta, according to electrophoretic or Cohn's fractionation, but can the body distinguish this beta from the other? In the diabetic rabbit after Tween 80, or in the animal with a tied common duct, no atherosclerosis occurs but endogenous cholesterol is high.*

DR. EDER: These abnormal lipoproteins actually are different chemically from the usual betas. Although they migrate electrophoretically as beta, by the Cohn method they come out in fraction IV+V+VI where alpha lipoproteins usually appear.

MODERATOR DOCK: *You do better on this than Dr. Gofman, or electrophoresis?*

DR. EDER: We do poorly.

MODERATOR DOCK: You get a figure, in liver cases, which suggests they are not going to cause the trouble which you expect in other cases with the high beta fractions.

DR. WILKINSON: I will put in a word there. We have been cooperating with the group at Bethesda for the past year, Drs. Anderson, Braggan and Boyle. While this work is still unpublished, it is finished and in the process of being published. They find all Sf* classes are not the same chemically. A man may have a certain amount of so-called Sf as 10:100 or 20:100 lipoprotein. His particular class of Sf molecules may be different from another individual who has the same type of lipoprotein in his blood, and in some people they even seem to change from time to time. It is well to remember that with the ultracentrifuge all they are doing is measuring physical constants, measuring how this particular molecule acts in the artificial field of gravity. It is not a chemical examination and unless you can prove that the content of these particles that you have distinguished because of one physical characteristic is constant, it is not proper to assign a certain chemical composition to all of them.

MODERATOR DOCK: I think the point that we are struggling to get at is this: If a doctor has blood cholesterol determined, this in itself is not much help. Cholesterol in a patient with obstructive jaundice may be extraordinarily high yet we know from all these studies that this does not predispose to atherosclerosis. This is purely endogenous, because the experiments with the animal show the endogenous production goes up about twenty-fold in animals within a few hours of tying the common duct. So you can raise the endogenous production of cholesterol to tremendous levels, and it appears in the blood stream in great quantities, but it does not precipitate out in the vessels of experimental animals and does not precipitate out in the vessels of people with obstructive jaundice. This does not help us much in practice unless we know how to alter the fractions by therapy. *The point we want to get out of Dr. Eder is how he is altering these fractions in the hopes that this might retard atherosclerosis.*

DR. EDER: We were impressed by the differences between young

* Svedberg unit = 10^{-13} cm./sec./dyne./gm.

males and females especially because of the known high incidence of coronary artery disease occurring in young males and its comparative rarity in young females. Also we were struck by the fact that as aging took place the difference between males and females was largely obliterated and many in the population became distinctly abnormal. Because of these differences we undertook the study of the effect of sex hormones on the lipoprotein pattern.

Patients were treated with large doses of estrogens (1 mg. of Estinyl or 15 mgs. of Premarin daily). In every instance alpha lipoprotein cholesterol increased. Beta lipoprotein cholesterol decreased in most patients. This change was greatest when the concentration of beta lipoprotein was increased. Under these circumstances the plasma cholesterol fell markedly. These changes were apparent by the first week and nearly maximal by the second or third. On stopping therapy the pattern returned to its original form rapidly.

In addition to changes in cholesterol distribution, estrogen administration resulted in alterations in cholesterol-phospholipid ratios in the beta lipoproteins. The ratios dropped markedly and this was apparent in the ratios for whole plasma.

The side effects of the estrogen therapy were distressing to many of the patients. Breast tenderness and impotence were often distressing. The psychic side effects, with feelings of lassitude and inadequacy were common. In an effort to eliminate these effects, combinations of estrogen and androgen therapy were attempted. The administration of androgen resulted in a prompt loss of the estrogen effect. We were unable to find a combination which would produce the estrogen effect on the lipids without the distressing side effects.

We have no data that would permit drawing conclusions as to the clinical efficacy of estrogen administration. It was impossible to determine changes in the frequency of anginal attacks in these few patients. We have no data as to the effects on survival. We do have one patient with idiopathic hyperleukemia and xanthomatosis who responded in a dramatic fashion. He was a mechanic with lesions on his hands which were so painful as to prevent his working. After one month of estrogen treatment his lesions disappeared completely. Six months after the cessation of estrogen treatment his lesions returned.

DR. KENDALL: What does he require in the way of dose?

DR. EDER: The same as the others.

MODERATOR DOCK: *Large doses with a large physiological effect? You are not using this except in occasional cases for short periods of time?*

DR. EDER: We have only used this as a research tool.

MODERATOR DOCK: *You don't feel it is applicable for the management of the disease?*

DR. EDER: I do not.

MODERATOR DOCK: *Dr. Adlersberg, you were one of the first to feed something to patients with familial hypercholesterolemia, hoping to control their disorder. What is your view now about modifying the disease by adding something to the diet?*

DR. ADLERSBERG: Various possibilities have been studied in this respect and the experience collected to date has not been too convincing. We started by giving "commercial lecithin" to some patients with hypercholesterolemia and we noted a lowering of serum cholesterol level in a few of them. I believe at this time that this was probably caused by the high concentration of plant sterols of this product which interfered with the absorption of cholesterol. The results were not consistent. Recently, various cholesterol analogues, such as sitosterol and dihydrocholesterol, have been used in experimental animals and in man to impair the absorption of dietary cholesterol. In the cholesterol fed rabbit–I have no personal experience with that–one can apparently prevent the hypercholesterolemia, which results from cholesterol feeding. If one uses any of these substances in man, the results again are not consistent; one may be dealing in man with different factors of intestinal absorption than in the herbivorous rabbit. On the other hand, one may have doubts whether diminished cholesterol intake will significantly affect the serum cholesterol level. The latter is the result of at least two factors, –of the intake of cholesterol, exogenous cholesterol, and the synthesis of cholesterol in the body, endogenous cholesterol. There is good evidence in the animal that diminished intake enhances cholesterol synthesis and vice versa. One could then reason that diminished intake in man could result in a secondary increase of cholesterol synthesis and thus counteract any effect that diminished intake may exert. The level of dietary fat is perhaps more important than the level of dietary cholesterol.

I would like to mention on this occasion that we have been studying, in collaboration with Drs. Hellman, Rosenfeld and Gallagher, of

the Sloan-Kettering Institute, certain aspects of cholesterol metabolism by using labeled cholesterol as well as labeled acetate. The purpose of the study was to compare the behavior of cholesterol derived as such from the diet with cholesterol synthesized endogenously from acetate. Acetate and cholesterol were available labeled with either radioactive carbon or radioactive hydrogen (H_3 or tritium). The studies of endogenous and exogenous cholesterol metabolism could be carried out separately or simultaneously in the same patient, using acetate labeled with one isotope and cholesterol labeled with the other.

Of the patients studied, three had normal plasma cholesterol levels and four had hypercholesterolemia (serum levels over 300 mg per cent); of the latter group two had xanthoma tendinosum, one xanthoma tuberosum and one nephrosis. The free and ester cholesterols of plasma and red cells were isolated separately in all the subjects. Analyses of urine and feces were included.

The specific activity of free cholesterol biosynthesized from acetate is at a maximum with the earliest serum sample and this activity declines rather rapidly. The tritium content of the ester cholesterol derived from acetate is at a minimum value with the first sample and then gradually rises until it intersects the free curve at approximately *three* days, after which the specific activity of the ester cholesterol exceeds that of the free. The behavior of cholesterol synthesized from acetate is the same whether the acetate is labeled with either tritium or C_{14}, and these data demonstrate that these two isotopes may be used interchangeably for the study of the conversion of acetate to cholesterol.

The plasma free cholesterol radioactivity originating in the diet (exogenous incorporation) is at a minimum value with the three hour sample. The specific activity then rises to a peak at about 1.5 to two days and afterwards slowly declines. The radioactivity of the "exogenous" plasma ester cholesterol is also at a minimum with the first sample, rises less rapidly than that of the free sterol, and reaches a peak value at about 2.5 days having intersected the free curve at about *one day*. After this crossover point, the ester specific activity is greater than that of the free for the duration of the period of experimental observation.

In patients who had idiopathic hypercholesterolemia (xanthoma tendinosum) the mode of rise of free and ester cholesterol, the points of intersection, the subsequent rate of decline, and the relative specific activities are virtually indistinguishable from those of normocholester-

olemic persons.

The findings in a patient with idiopathic hyperlipemia and xanthoma tuberosum and in another patient with nephrosis, whose sera showed marked lactescence, differ in *two respects* from those obtained in subjects with normal cholesterol levels and from idiopathic hypercholesterolemia in the xanthoma tendinosum type. The free cholesterol synthesized from acetate, although at a peak value with the initial sample, declines more slowly during the first few days of observation, so that the rising specific curve of ester cholesterol derived from acetate does not intersect the free curve until about the *sixth day*. This is in contrast to the behavior of all prior patients studied with acetate, in that the point of intersection previously occurred at two to three days. The second difference is to be noted in the early portion of the specific activity curves representing the plasma cholesterol arising from dietary sterol. The ester cholesterol specific activities are initially at a higher level, rise more rapidly, and exceed those of the free throughout the experiment. Since the free specific activities are always below the ester values, *the two curves do not intersect.*

These observations suggest biochemical differences between idiopathic hyperlipemia (lactescent serum) and idiopathic hypercholesterolemia (clear serum). Aside from these differences in the early portion of the two sets of curves, the latter parts of the curves show a parallel rate of decline of cholesterol radioactivity whether of endogenous or exogenous origin. In addition, this latter rate of decline is similar to that observed in other patients with hypercholesterolemia as well as in the subjects with normal plasma cholesterol levels.

The recovery of administered radioactivity in feces by the fourth day varied from 13 to 50 per cent. A much smaller proportion of the administered material was excreted in the urine during the same time interval; this varied from 0.35 to 1.75 per cent.

Samples of expired air were collected at frequent intervals from the patients who received C_{14} cholesterol. Carbon dioxide isolated from the breath, failed to show radioactivity. Far less than 1 per cent of the administered dose could have been detected in the expired air by this method.

The curves for plasma free and ester cholesterol derived from the diet decline at similar rates after reaching their maxima and are comparable to the behavior of plasma cholesterol synthesized in the body from

acetate. The ester specific activity remains higher than that of the free because the free cholesterol is continually being diluted by non-labeled cholesterol from the diet or by endogenous synthesis.*

These data suggest that dietary cholesterol and cholesterol synthesized in the body mix indistinguishably after the initial processes of absorption and distribution have been completed. If the body distinguishes between endogenous and exogenously derived cholesterol, this could seemingly only occur during the immediate period after absorption or synthesis. The radioactivity values of tritium and carbon-14 obtained in the same sample of cholesterol decline at the same rate.

During the early phases of absorption and/or synthesis biochemical differences in lipid metabolism in various pathological conditions associated with hypercholesterolemia are suggestive.

MODERATOR DOCK: Our time is almost up and we must try to answer yes or no to some questions submitted by the audience.

The first question I would like to ask each member of the panel in turn would be this:

Is there any point in modifying your diet in order to modify the course of arteriosclerosis? Dr. Kendall, would you be able to say anything at all if someone asked you this question? Just a yes or no, there either is or isn't a point in modifying the diet.

DR. KENDALL: I would say yes.

MODERATOR DOCK: *You think the diet might have some influence. Dr. Adlersberg, what do you think?*

DR. ADLERSBERG: I would say yes. I would like to add, however: Don't eat too much, keep your weight at the normal or at the ideal-weight-level; if you happen to be stout, weight reduction to a normal weight level is justified and desirable.

MODERATOR DOCK: *Dr. Eder, what do you tell your patients?*

DR. EDER: I say do as I say, not do as I do.

MODERATOR DOCK: *That is reasonable. The primrose path you follow is not for the patients. You tell them to modify their diets?*

DR. EDER: To lose weight, yes.

MODERATOR DOCK: *To lose weight, not to be concerned about anything but total caloric intake, keep the balance of food where it was?*

DR. EDER: Yes.

MODERATOR DOCK: *Dr. Wilkinson, what advice do you give?*

* The data discussed above will be published in the Journal of Clinical Investigation, January 1955.

DR. WILKINSON: I advise that they lose weight and preferably be under the ideal weight at thirty. We do not take our age tables past thirty. We do not alter the amount of fat or cholesterol that they eat.

MODERATOR DOCK: So I think you are all now fairly clear what the panel thinks about diet. They want to make the problem harder for the farmer and the surpluses to be bought with federal funds even larger. Just less of everything and no specific dietary management.

Dr. Kendall, have you had any experience with blocking agents, as far as man is concerned, sitosterol or dihydrocholesterol?

DR. KENDALL: None.

MODERATOR DOCK: *Dr. Adlersberg, have you had a chance to try this on a patient?*

DR. ADLERSBERG: I have had very limited experience with that and it was not satisfactory.

DR. EDER: No.

MODERATOR DOCK: *Dr. Wilkinson, you have been working on this. Is there any hope in this field?*

DR. WILKINSON: We have done considerable metabolic work with sitosterol, which was the first of the vegetable sterols to be reported and we found that in man it had no demonstrable effect. Some of our cases now go up to thirty-six weeks.

MODERATOR DOCK: *So there has been no change and you have been having Gofman fractions and other things run on these patients?*

DR. WILKINSON: We did lipo fractionation. We have had patients in the metabolism wards of the hospital for periods up to forty days who went through three periods. During the first period they were put on a diet containing less than one-quarter of a gram of fat per day. Then we added fat to that diet and then we added the sitosterol in the third period. We won't have time to go into the many things that we did find out on the diet but I think it is safe to say, Dr. Dock, sitosterol is a failure in our hands.

MODERATOR DOCK: In man. What happens in the chick and rabbit is not important.

DR. WILKINSON: Very few of our patients have long ears!

MODERATOR DOCK: That is the trouble, and they don't come out of eggs, so here is another hopeful lead, developed by experimental methods, which so far shows no human application. *I think everybody on the panel is probably agreed that there is no use feeding inositol,*

choline, methionine, lecithin or any of these so-called lipotropic agents. Is there any dissent from that?

DR. WILKINSON: I would like to add thyroid unless the patient is hypothyroid.

MODERATOR DOCK: That is, endocrine therapy is only for the endocrine patient—which the patient would need if he did not have anything wrong with his cholesterol. The blocking agents so far show no promise in man. As to therapy with female hormone, the inconveniences outweigh anything except the most difficult situations, such as that of the man whose hands were crippled by his disease. So that the only rational hormone therapy is that which you would give the patient if he did not have any trouble with his vascular system. I think that is the sense of the panel. *Now the problem comes up, has anyone had any experience with heparin that would lead him to believe this would have any use in atherosclerosis?*

DR. WILKINSON: I have had no experience. Last year I worked with Dr. Boyle who was using heparin in some of the joint conditions. It is a difficult drug to use. It does have some effect on certain of the lipoprotein fractions but unfortunately, the patient on whom he was getting the best effect had another coronary attack and died while he was getting the heparin.

MODERATOR DOCK: *Dr. Eder!*

DR. EDER: We have made no *clinical* observations with heparin. We studied the plasma lipids following heparin administration.

MODERATOR DOCK: *Which are not striking?*

DR. EDER: They are very striking.

MODERATOR DOCK: *In the beta and alpha fractions too?*

DR. EDER: The neutral fat in beta lipoproteins decreases and the cholesterol and phospholipid decrease to a lesser extent.

MODERATOR DOCK: *So there may be some effect. Whether this affects atheroma formation you don't know?*

DR. EDER: We don't know.

MODERATOR DOCK: *In animals, Dr. Kendall, is there any evidence that heparin will retard atherosclerosis?*

DR. KENDALL: We have never carried out an experiment long enough. All of our observations have been on the short time effect of heparin.

MODERATOR DOCK: *And you don't know of any other observations that would indicate that this might be helpful therapy?*

DR. KENDALL: No.

MODERATOR DOCK: *So it appears that our panel consists largely of therapy nihilists. They have no treatment for the management of atherosclerosis, in blocking agents, hormonal therapy or in lipotropic agents. They feel that nothing has been shown in man about the role of cholesterol in the diet so that you don't need to lower the cholesterol fraction of your intake. All the members of the panel agree to this?*

DR. WILKINSON: Yes.

MODERATOR DOCK: *Dr. Kendall, you do too?*

DR. KENDALL: That is all.

MODERATOR DOCK: I am afraid therefore only the moderator persists in sin and thinks the low incidence of atherosclerosis he sees when he goes visiting in other countries as compared with that in Brooklyn is related perhaps to the difference in cholesterol intake. It is very difficult for the moderator to get out of his thick head the idea that something which is harmful to a number of different animals is entirely harmless to man. Low protein diets with high cholesterol content produce very high blood cholesterol in dogs, as Freeman and Li showed long ago. Those dogs had their thyroids functioning. My own feeling is that a high protein, low cholesterol, low fat diet is as reasonable as any that could be recommended. I suppose none of you want to cut the protein content of the diet down if you are reducing weight. Again you want to keep the protein levels fairly high.

DR. WILKINSON: I would rather wait a year to talk to you about that. We have some experiments in progress. We cannot draw any conclusions at this point.

MODERATOR DOCK: *You don't know whether high protein is good, bad or indifferent?*

DR. WILKINSON: We suspect that a very high protein is not good.

MODERATOR DOCK: *What would that be, 150 grams?*

DR. WILKINSON: Probably not good over 125 grams, and we want to try at various levels of protein and find out where we seem to get the best results.

MODERATOR DOCK: *In terms of blood cholesterol levels?*

DR. WILKINSON: No, in terms of blood cholesterol levels and ultracentrifugal amounts.

MODERATOR DOCK: *Reasonable protein but not a very high protein diet you think is more sensible?*

DR. ADLERSBERG: I just wonder whether we should finish on this note that the cholesterol content of the diet, and/or the fat content, or the calories as such, are or are not "the factor" in human atherogenesis. I do not believe that our present knowledge permits a definite opinion. It is one thing to conduct metabolic studies in man or animals over a period of 30 or 60 or 120 days and it is another thing to eat large quantities or small quantities of cholesterol and/or fat over a lifetime or through generations. Although a low incidence of atherosclerosis has been observed in the Far East and in Bantus in South Africa, I feel that that might be caused by the very low calorie intake as well. The shorter life span of these people naturally interferes with the development of atherosclerosis. I don't believe we have enough information so far to decide this question definitely. Extensive statistical studies are needed.

MODERATOR DOCK: There we are at the moment. I think some of you saw during the last year, in the J.A.M.A., a striking difference in the vascular state of the North Koreans who were autopsied after being killed in action and the members of the United Nations forces who were autopsied after being killed in action. The most striking of all I think is the difference in the incidence of atherosclerosis in young members of the American Army and Navy in the last fifteen years and the experience in the British Army and Navy. While we were accumulating hundreds of cases of young men dead of coronary disease, the British Army and Navy had a low incidence. They finally dug up thirty-nine fatal cases in men under thirty-five, from their pension office figures covering many years. So there is some difference in the incidence of atherosclerosis in people eating different diets but just how the diet affects the situation we don't know. At the moment the only thing we can be sure about is that if you put the patient on a low calorie diet, it will be very difficult to put him on a high fat and high cholesterol diet. You come out with a sort of common denominator, a diet which is low in calories, reasonably good in protein and water soluble vitamins and almost certainly this will be relatively low in fat and cholesterol. It does not need to be made unpleasant to get the fat and cholesterol too low.

DR. WILKINSON: I don't know how you can say a fattening diet is fat faulty. Dr. Ansel Keyes once said a little bit of grease goes a long way on the outside of food. People who become obese usually eat

large quantities of bland carbohydrate and flavor only with fats. On taking a number of dietary histories both with Dr. Newburgh at Ann Arbor and later here, I found that many obese persons are not large fat eaters for the most part. Most of their calories come from bland carbohydrate. Take a big Idaho potato and put a piece of butter on it, but figure out the relative calories between the two.

MODERATOR DOCK: Obesity we still don't know the answer to. *An enormous number of animals are fattened for the market and yet none ever get atherosclerosis. I believe that is right, Dr. Kendall?*

DR. KENDALL: I understand that they are beginning to find that atherosclerosis is a common disease in hogs fattened for the market.

MODERATOR DOCK: *On what sort of diets, corn? They did not get cholesterol.*

DR. WILKINSON: Carbohydrate.

MODERATOR DOCK: Then we must close with the thought that obesity, even that due to carbohydrate feeding, may predispose mammals to atherosclerosis. In spite of the evidence that obesity plays no part in causing coronary disease in men under forty-five years old, the Panel emphasizes avoidance of obesity as the only effective prophylaxis. The moderator would prefer a liberal protein, low cholesterol diet, and we all await further light on this subject.

OUR HERITAGE FROM WILLIAM OSLER*

STANLEY E. DORST

Dean, College of Medicine, University of Cincinnati

ON THE first day of January 1920 in the ancient chapel of Christ Church Cathedral, Oxford University, a distinguished assembly of Britain's great gathered for the funeral services of Sir William Osler. Not only the great were there. The students and the simple people who compose the more humble echelons in every great University town also crowded the aisles for their friend, the beloved physician, was dead.

The quiet which brooded over the lovely spires of Oxford spread widely. It crossed the Atlantic and was acutely sensed over our great continent shrouding the United States and Canada. A medical student, returning from his Christmas holiday on the second of January, was suddenly aware that something of consequence had occurred. That was before the day of radio but even so my family physician in the small Ohio city in which I then lived had called to tell me in a grief-stricken voice that William Osler was dead. Osler had been his teacher at Johns Hopkins. I returned to school to find my own Professor of Medicine quite broken, for Roger Morris had been Osler's resident and later had served under him in the Hopkins Department of Medicine.

So, as it happened, I never saw him, never heard him speak, but William Osler's character and personality have exerted a direct influence down through the years of my life which has been equalled only by a very few persons with whom I have had the fortune of intimate contact. His textbook of medicine through which he said he came in contact "mind to mind with the profession" was the student's bible in my day and his published essays and lectures I have read and re-read whilst Harvey Cushing's magnificent biography has been constantly at hand since it was published in 1925.

But, as was inevitable, there has come a generation "which knew not Joseph." Medical students of today and most physicians under thirty years of age have little knowledge of Osler's tremendous contribution

* Presented by invitation before the Section on Historical and Cultural Medicine at The New York Academy of Medicine, May 12, 1954. Manuscript received July 1954.

to medical education in the United States and they are quite unaware of the truly great debt of gratitude which they owe to him. *Aequanimitas* they all have had through the wise generosity of one of our great pharmaceutical houses, and William Bean's little labor of love, Osler's "Aphorisms" has been available since 1950, to say nothing of Cushing's two volumes; but they are so seldom read. We are all too busy with "important things."

I will speak about Osler tonight, not as a medical historian for I would be poorly cast in such a role, but rather as a physician who would acknowledge his own debt, and, if my discourse runs largely into the field of medical education, it is because I hold today, however unworthily, the office Osler once graced, the Presidency of the Association of American Medical Colleges. The business of that Association is medical education and not one of its long list of Presidents has measured up to the accomplishments of William Osler in this field.

I am asking you to pause and journey back with me some sixty years. The year will be 1892, the place, the University of Minnesota, and the occasion marked the dedication of the new medical buildings at that University. The speaker was Dr. William Osler.

What he said might well have disturbed many persons in his audience, because on that day, devoted to the opening of a new medical school, Osler elected to speak quite deliberately of the fourth-rate position occupied by the American medical schools when compared with the advanced university schools of Germany, Britain and France. He declared that "in this eminently practical country the teacher of medical science has not yet received full recognition, owing in part to the great expense connected with his work, and in part to carelessness or ignorance in the public as to the real strength of a nation." As a result schools of medicine had not developed as faculties of universities except in rare instances but instead they were still proprietary schools of a type to be countenanced only in a frontier economy. He said quite frankly: "To equip and to maintain separate laboratories in the sciences of Anatomy, Physiology, Biological Chemistry, Bacteriology, Pathology and Pharmacology and to employ skilled teachers, who shall spend all their time in instruction and research, requires a capital not today at the command of any medical school in the land. There are fortunate schools with two or three departments well organized, not one with all." And of the one hundred and fifty-odd medical schools in the nation

on that day the fortunate ones with two or three departments well organized could have been counted on the ten fingers and with fingers to spare. That was in 1892, within the lifetime of many persons in this hall today.

Dr. William Osler was then forty-three years old. He had recently been appointed Professor of Medicine at the new Johns Hopkins Medical School which was destined to exercise so dominant an influence upon the future of American medical education. I am not guilty of exaggeration when I refer to our "heritage from William Osler" because, even in that group of brilliant teachers who appeared on the American scene as his contemporaries, he stands out pre-eminent, the apostle of modern medical education. No man of his time was more earnestly concerned with the problems of the medical schools, no man was more articulate in expressing their needs, and no one struggled so constantly to remedy their defects.

The crusade which was already well under way when Osler spoke at Minneapolis may be followed in his published addresses. His analysis of medical education became more precise in the decade leading to the turn of the century and repeatedly he emphasized three great defects. The first was the need for the development of true university schools, the second for the intimate integration of medical school and hospital so that the wards should become the true laboratory of the clinical departments, and the third need was for the active prosecution of research in every department in each medical school. All of these things had been accomplished abroad—in the United States they were sadly lacking.

To understand these three needs we must try to look at them as Osler saw them because their fulfillment was to be the legacy of William Osler to medical education, and we must comprehend the inheritance in order to evaluate the manner in which we have invested our heritage.

In the nineties, as I have said, there were but few university medical schools. The great majority were independent proprietary institutions, only a few with nominal university affiliations. It was quite clear to Osler that the schools which were most vital and progressive were those with close university ties, and his constant plea was for a tightening of the bonds between medical school and university throughout the country. He was not unmindful of the important contribution made by the proprietary schools during the first half of the 19th century but

he argued quite correctly that these schools, so numerous and so very inadequate, the product of a crude frontier society long gone, must merge and then find sanctuary in the shadow of a university or they must close their doors.

Medicine, at the end of the century, had indeed entered a new era. The epoch making discoveries in bacteriology, physiology and pathology; the birth of immunology, hematology and biochemistry were to change the entire complexion of medical thought. New techniques of diagnosis and treatment, the results of those discoveries made largely in the research laboratories of Germany, France and England, were to find practical application in the hospital and at the bedside. The only sound way to introduce them was through the schools of medicine, and that would involve nothing less than a complete reconstruction of American medical education: a vast program of physical expansion to provide laboratories and other facilities, and the training of a small army of teachers and investigators to provide pre-clinical faculty to teach the medical sciences. And while this was being accomplished it would be necessary to change drastically the educational requirements prerequisite to the study of medicine, for the proprietary schools in most instances accepted students directly from high school. These schools had served their purpose and their day was passing; it was passing because the demands to be made upon them were overwhelming. To replace them Osler led an ever-growing band of physicians devoted to the conviction that "thoroughly equipped laboratories in charge of men thoroughly equipped as teachers and investigators was the most pressing want . . . in the medical schools of this country."

This was the first great need, that the schools be remade so that they might teach medicine as it was being taught abroad and faculties, literally, must be created where they did not exist, to carry forward teaching of medical science.

As a corollary to this first need, that the schools should be expanded as an integral part of the universities, comes Osler's second plea: that the hospitals in which the teaching of clinical medicine was to be done should be intimately associated with the schools and that medical students should be taught in small groups on the hospital wards and in the dispensaries. He expounded the great value of the clinical clerkship which does not merely admit the student to the amphitheatre and lecture bench on sufferance, but instead brings him into the hospital as an important

member of the physician-student team. This team was to be organized upon the basic assumption that all members from medical student on through house staff, attending staff and service chief were students together, differing only in degree of experience and wisdom. Necessarily the opportunity for graduate training in the hospitals should be greatly expanded through the development of intern and residency programs.

Less than fifty years ago they were far ahead of us abroad in this correlation of medical school and teaching hospital. We were still lecturing and demonstrating to large numbers of students in crowded lecture rooms and Osler was pleading for small groups, each with a competent instructor, studying the actual, daily progress of disease on the hospital wards and in the out-patient dispensaries. What he wanted seems obvious enough, and reasonable too, for it is the essence of sound clinical instruction, but a profound change in point of view involving medical teachers, visiting physicians, hospital authorities and university authorities was necessary to make it possible. It was the last of Osler's three "needs" to be adequately satisfied.

The significance of his efforts in this direction is attested to in the dedication of Harvey Cushing's monumental life of William Osler where we read:

"To Medical Students

> "In the hope that something of Osler's spirit may be conveyed to those of a generation that has not known him; and particularly to those in America, lest it be forgotten who it was that made it possible for them to work at the bedside in the wards."

When he left Johns Hopkins Medical School, Osler was not convinced that he had accomplished more than a local reform which changed the nature of clinical teaching in a scattered half dozen medical schools, and he had devoted more than fifteen years to his crusade in behalf of medical education. However, from those schools, acting as secondary foci, the influence spread so that Cushing's dedication, written in 1925, was entirely appropriate.

When the university medical school and the teaching hospital should be consolidated into an effective educational unit the stage would be set for the realization of a third development which would vitalize and nourish American medicine. Osler insisted that the new schools of medicine must appreciate the dual function of a university, namely, to

teach and to think. The idea was quite simply expressed in his own words: "What I mean by the thinking function of a university is that duty which the professional corps owes to enlarge the boundaries of human knowledge. Work of this sort makes a university great, and alone enables it to exercise a wide influence upon the minds of men." Research must become a responsibility of equal rank in its importance with teaching, and then the third great "need" which Osler expounded would be satisfied.

He realized the error in the point of view then widely expressed by the men of "common sense," which maintained that a few select schools should be equipped and staffed for the pursuit of investigative work while the great majority would continue to train practical doctors much as they had been doing. Only a faculty which contained a leaven of men seriously interested in widening the field of knowledge could be progressive, and to remain static in this new era of medical education was to deteriorate. Repeatedly he called attention to the obvious significance of what had happened in France, Germany and Britain and he insisted that the recent developments in medical research abroad were vast enough to challenge every faculty of medicine in this land.

I hope you will forgive me for taking so much time to recall what many of you have long known, but we must neither permit time to dull the great importance of William Osler's analysis of medical education, nor forget his untiring efforts to bring the urgent need for adequate corrective measures before both the physicians and the intelligent laymen of the nation. A few years later A. N. Whitehead was to make his striking plea for the necessity of "the habitual vision of greatness," for, he said, "if we are not great it does not matter what we say or what we do." I submit that Osler did indeed hold the habitual vision of greatness in medical education before the profession in the United States; he did not permit them to ignore or to forget it. This was his legacy to us.

In 1905 he left Johns Hopkins University to become Regius Professor of Medicine at Oxford, and soon thereafter became Sir William and the second living Canadian Baronet. He knew he was leaving a positive movement and an active ferment in certain American schools of medicine. He could not know how amazingly that movement would accelerate. The time was ripe, of course, or Osler's would have been a vain voice among that tragic host of wise men who lived before their time. The Johns Hopkins conception of medical education succeeded

beyond the most ambitious expectations of its proponents; the ideas it developed and the men it trained spread through the medical schools in spite of the bitter resistance of entrenched reactionaries. The stern admonition of President Eliot of Harvard leveled at the faculty of medicine in that university a decade earlier, that they promptly bury the dead past and put about the business of setting their house in order, had echoed through the country. The time was indeed ready and everywhere sincere and competent men were carrying forward the crusade which Osler had preached for almost twenty years.

Ask a well-informed physician what brought about the great renaissance in medicine in this country and today the chances are about ten to one that he will reply, "The Flexner study and the Report of 1910." Time as it passes folds cause and effect into each other so that true relationships are often lost. Nothing should be said to detract from the importance of the Flexner study, but it must be remembered that the famous report was both an ending and a beginning. It marked the end of a period of intense preparation which made the nation ready for the findings of Dr. Flexner, and permitted development of an effective program in medical education which implemented the report. It marked the beginning of an era of such rapid progress that within three decades the medical schools of America had not only drawn abreast of the schools abroad, which Osler had held as the almost impossible goal, but had indeed surpassed them, providing the most effective training for all the young physicians of a nation that the world has seen.

This all-important period of preparation to which Osler contributed heavily saw an entire profession so dedicated to self-improvement that the Council on Medical Education and Hospitals, created a year before he left Johns Hopkins, could go to the Carnegie Foundation for the Advancement of Teaching, with the full approval of the House of Delegates of the American Medical Association, and propose an objective study and report which, as was well known in advance, would close sixty per cent of the nation's medical schools should it prove successful. In order to keep the record clear one must emphasize the fact, frequently ignored, that the implementation of the ideas implicit in Osler's crusade was, and had to be, carried forward by the organized medical profession. The Flexner study was conceived and proposed by the A.M.A. and its membership stood ready to support the findings even though they destroyed almost two-thirds of the institutions which had

produced them—a truly unique accomplishment which tested the effectiveness of that period of preparation.

There is not time this evening to tell the story of that amazing rebirth of medical education and subsequently of medical practice which marked the first quarter of this century. Indeed there is no need to do so since we are all familiar with the story and many of us have been permitted in a small way to contribute to its writing. Though the full flood of this progressive movement has been reached there has been no ebbing of the tide; the schools of medicine have not become smug and complacent.

Since it was Osler who constantly measured our deficiencies by contrasting the American schools with those abroad, perhaps we, in turn, might measure the American achievement by reversing the process, and look at our schools through the eyes of a modern and competent foreign observer. Robert Debré, the distinguished professor of Pediatrics of the Faculty of Medicine, University of Paris, has made several extended trips to the United States since the end of the war for the purpose of studying medical education in our country. Recently he published an account of his impressions in an important magazine, the "Revue de Paris," thus choosing to speak widely to the people of France through a lay journal, rather than limit his message to the medical profession. I wish to quote from the French publication.

"Each of these great Universities of America has a School of Medicine. They are richly financed . . . they are designed to instruct relatively small classes of students (as judged by European standards) winnowed from numerous candidates, whose intellectual and moral qualifications are taken into careful consideration in the delicate matter of their selection. Each stage of promotion in their select schools involves sound basic education . . . Young men reared in these colleges understand the technique of therapy which they themselves have practiced, they know how to discuss problems of biological chemistry such as come up in clinical practice, they base their ideas on sound physiological foundation. Our students cannot do the same in spite of the efforts of excellent professors. But, to tell the truth, they cannot be compared. In this domain as in others the differences are too great, the organization and the resources too dissimilar. There is no resemblance between these peerless American Schools of Medicine and ours."

And only fifty years earlier Osler was speaking in almost identical

words, only the implication was reversed. Professor Debré goes on to say: "It is not only the importance of the laboratories and their good equipment, their adequate resources, that must be emphasized. The spirit which animates this university corps and the type of life which is organized in these schools cannot be considered with too much attention. . . . The free intercourse, less formal and less hierarchic than in the Old World, of these men passionately interested in their task really gives the impression of a balanced team. The whole group spends its day in the hospital and laboratories. The hospital, associated with these medical schools, is an integral part of the organization. . . . The School of Medicine is one mingled with the hospital and there is none of the fragmentation from which we suffer in France. The fusion, material and spiritual, of the teaching staff with the research staff and the hospital staff is total. When shall we arrive at this solution, the only logical and satisfying one!"

I hope you have noted how strikingly though quite unconsciously Professor Debré highlighted the fulfillment of Osler's three great needs in these passages I have quoted from his critique of our medical education. His evaluation is not unlike many others which have come from abroad in recent years and I believe one might conclude that, judged by the evidence of foreign medical educators, we have indeed given a good accounting of our trusteeship, have promoted our heritage so well that it has yielded a golden harvest. Surely Osler would have no criticism but instead only astonished praise for the way in which we have carried forward his endeavors to accomplishments which he could not have anticipated in his most optimistic moments.

But, of a sudden, doubts creep in and now I am not so certain. The material, the physical and the intellectual achievements; the organizational integration of hospital, university and research laboratories, all would meet with Osler's most enthusiastic approval were he to pass judgment on our efforts today; but then, I fear he would somewhat sadly suggest that in the face of this truly great achievement we seem to be in danger of neglecting one of our first obligations, namely, to do our utmost to bring to each student some awareness of the essential spiritual nature of our profession.

To William Osler the practice of medicine was not only an interesting way of earning a living; it was something far more comprehensive—a way of life. This, in truth, is what the great professions have always

been—as long as they remained great. It is the common denominator which is to be found in everything Osler wrote and worked for, and nowhere is it more clearly expressed than in the last four addresses which he delivered before leaving the United States. Together they constitute his valedictory to the profession in this country. Even the titles are significant—"The Fixed Period," "The Student Life," "Unity, Peace, and Concord," and finally, only a few days before sailing, the exquisite little "L'Envoi."

For him the ultimate goal of all education was not technical competence, not accumulated knowledge, but the development of wisdom. What is wisdom in the physician? To the best of my knowledge Osler never spelled it out but I believe his meaning is clearly apparent; wisdom is knowledge which through experience is transmuted into understanding—and that is what we are in danger of losing in the excellent medical education of our day.

Paradoxically our very success in eliminating the three great defects which Osler deplored was a factor which contributed to the faults we now recognize and hope to correct. The brilliant interval which we might identify as the post-Flexner period of medical education saw the rapid shift of emphasis from the art to the science of medicine. Precise methods of diagnosis, new understanding of the nature and cause of disease growing out of an expanding scientific knowledge, and the development of specific therapeutic agents, not dreamed of even twenty-five years ago, have all exerted a profound influence upon the pattern of medical practice.

Increasing specialization which is ever the close companion of advancing technology has made it apparent that four years of undergraduate study even with a year of internship added did not allow adequate time for the mastery of any branch of clinical medicine. So the residencies appeared, grew and spread. The residency system is undoubtedly our outstanding achievement in clinical medical education. The idea came from abroad, like so many others, but we have developed it into a unique institution. However, the residency has accelerated the over-production of specialists and increasingly institutionalized our physicians.

The truth is that an objective evaluation of our progress will reveal the faults of our very virtues—and we know it. Any thoughtful person can list a disquieting number of defects, such as the increasing length

of time required to prepare a physician for practice; the too-apparent over-emphasis upon specialization; the practice of tempering the young physician in the press of institutionalized patients rather than in the field of active practice; and, growing out of this era of scientific influence, the distressing fact that too often our students have been trained to study disease rather than to understand the problems of a sick human being.

Many of us who teach medicine have become aware of our shortcomings. We are beginning to realize that specialization breeds technicians, not physicians in the Oslerian sense, and more has already been done to counteract our faults than is widely realized. The great upsurge in psychiatry in the medical schools has resulted in the beneficent intrusion of psychosomatic concepts into every special discipline, thus bringing into focus the sick patient rather than an exhaustive list of laboratory tests. The increasing emphasis now placed upon environmental medicine, and the widening effort to help the student become familiar with the impact of sickness in the atmosphere of the home, all are evidence that the medical schools are again becoming aware of the social responsibilities implicit in their job. In school after school the curriculum is being subjected to critical study. Experiments large and small are appearing throughout the land, and in the newly established Teaching Institutes of the Association of American Medical Colleges the medical faculties of the nation have embarked upon a program of critical cross fertilization which is unlike any previous experiment in the scope and ambition of its purpose.

What we now attempt to do is to humanize the competent technician produced by the brilliant post-Flexner period of medical education so that we may realize the full measure of William Osler's legacy to the medical schools. Several years ago I tried to express the ideal we strive for and I believe I can do no better than repeat what I said then: In his service the professional man must act in a three-fold capacity. He must appear simultaneously as skilled technician, as sage and as priest. In no professional role is this trinity more fully illustrated than in that of the physician. Knowledge is needed and often most detailed knowledge sharpened into tempered skill; but that is not enough. Wisdom must be there to condition knowledge; but that is not enough. Understanding must guide skill and humanize wisdom; then only do we see the complete physician.

SECTION ON MICROBIOLOGY
and
NEW YORK PATHOLOGICAL SOCIETY

Papers Presented at the Combined Meeting
At The New York Academy of Medicine, January 20, 1954

GERMFREE RESEARCH:
A BASIC STUDY IN HOST-CONTAMINANT RELATIONSHIP

I. *General and Theoretical Aspects of the Problem*

JAMES A. REYNIERS and PHILIP C. TREXLER
Lobund Institute, University of Notre Dame, Notre Dame, Indiana

The subject of host-contaminant relationship has many ramifications, which may be focussed where the host and its contaminants first meet. Such a point of view assumes that host and contaminants can be recognized as distinct biologic entities and their association as a dual biologic system is accidental.

If the focal point of discussion is the first meeting of host and contaminants, then it is possible to project a plan of investigation within the framework of a thesis. This thesis is that the pure culture concept may be extended to the animal and on this basis applied to problems in biology and medicine of which the host-contaminant relationship is one. Such an approach is long-range, basic in nature, and exploratory by intent but with advantages of logic and continuity. It presumes that the animal may live as a pure culture free from contaminants.

The conventional animal, after birth, may be considered as a dual biologic system consisting of host (animal) and contaminants (bacteria, worms, ectoparasites, viruses, protozoa, etc.). The investigator may describe this dual system as such and he may, for experimental purposes, add or subtract one or another contaminant, but he has no control as far as contamination in a general sense is concerned. Thus, the conventional animal at any point in its life is the product of continuing accidental microbial experience. As an experienced host, therefore, the conventional animal does not satisfy the demands of the pure culture concept. Unless some logic other than this concept is accepted as the basis for an experimental approach to the problem of host-contaminant relationship, increasing dependency must be placed on the naturalistic approach which is essentially descriptive. The natural approach is foreign to the experimental approach which is essentially artificial, deals with but a single variable, and depends on models to examine or create a phenomenon with the ultimate aim of controlling it to whatever ends are desired.

If the pure culture concept is to be extended to the problems which face us with respect to the host-contaminant relationship, the "host" must be reared free of contaminants, after which they may be brought into association with each other as pure units. Thus, the degree of experimental control necessary to the broad thesis is satisfied at least in theory. In practice, if the host is to be freed from its contaminants, this must be done at the most propitious stage in its life cycle and subsequently reared so as to prevent recontamination.

The host and its contaminants may become associated either 1) before or 2) after

birth or hatching.

1. *Contamination of the host before birth:* The animal, as such, exists first in the fertilized ovum which develops into an embryo and later into the entity capable of an independent life. The period of development prior to birth is one of preparation for independent life during which the animal is endowed with the cells, tissues, organs and systems necessary for carrying on this life and for meeting environmental threats of which microbic contamination is one. It is quite possible that a contaminant may exist as part of the male and female gamete, or at least in this environment in which the gametes develop and in which the fertilized cell grows toward independent life at birth. If this is the situation, then a high degree of synergism must exist; otherwise the association would be reflected adversely during development of the animal. It is, of course, entirely possible that a contaminant may exist as a true symbiont necessary to the economy of the gametes or the developing embryo. This will depend on how the term contaminant is defined and what connotations are implied. If a contaminant is taken to mean life other than that produced by the host protoplasm, the problem is somewhat simplified since there now remains only the problem of identifying this suspected life entity. Entering into this definition is the possibility that genes may act in a foreign cell to its detriment, i.e., changing the course of its development and acting as a virus. These things may call for new definitions; but, if true, such definitions must wait new evidence. On the other hand, viruses may exist as contaminants in the environment of the gametes and remain quiescent until such a time as they can become active in the embryo or animal. Finally, the probability exists that contaminants may pass through the mammalian placenta, or into the egg from the genital tract, and hence grow in the embryo without affecting it, or develop at a later date in the newborn young.

The animal must develop systems and mechanisms during the embryonic period to resist or adapt to contamination at birth and as a fertilized cell these systems may not be present. Moreover, virologists have taken advantage of the embryo to grow viruses in pure culture without contamination. The fact of the matter is that most normal animals develop toward independent life in a protected environment. It would seem illogical that such shielding would not exist while the animal is assuming identity and developing the necessary means for conducting independent existence. As an embryo, it is certainly not capable of meeting the threat of the external environment, much less carrying on independently in this environment. The normal animal at birth shows little detrimental effect due to contamination in the prenatal state. If a contaminant exists, it does not grow in competition with the healthy embryo.

Thus, even though there is a possibility that this animal and contaminants may meet before birth, there are still two separate entities involved and so it should be possible to separate one from the other.

2. *Contamination of the host after birth:* The second point at which the animal and contaminant may meet is at or shortly after birth or hatching. This is fairly evident for the external environment contains varieties of living forms, many of which are capable of growing in or on the animal. Moreover, experience has shown that the animal after birth becomes a host for contamination without exception. These contaminants are in the external environment, not in the animal before birth.

If the two possible points of contamination are considered, it is clear that any start toward separation of the animal from the contaminants must be made at that point where the animal is capable of independent life, i.e., near or at birth. It is possible to eliminate contaminants from the external environment and, if necessary, to devote attention to the elimination of internal contaminants when they can be demonstrated. Unless the threat of contamination at birth is eliminated, the latter problem is impossible of solution if the animal, and not the embryo, is the experimental unit. There is one other possible path to follow and that is to eliminate the contaminants at some point in the life of the animal after birth and after it is conventionally contaminated, but a little consideration will reveal the manifest

difficulties of this situation. Not only must the contaminants within the animal be eliminated but also those in the environment as well. Moreover, this approach complicates the problem tremendously with respect to the possible contaminants brought in with the germ cells, or added to the animal from its mother during intra-uterine life or from the egg during external incubation of the oviparous embryo.

It is the animal taken at or shortly before birth and reared in a sterile environment which we presently call "germfree"—a term which has the sanction of usage. We define a germfree animal as one which is free from all life other than that produced by its own protoplasm within the limitations of available methods to detect living forms. This animal may be considered free from bacteria, yeasts, molds, Rickettsiae, viruses, protozoa, worms and ectoparasites. Further, we mean that not only is the animal free of these contaminants so far as we know, but so is the environment in which it passes its life and goes into successive generations. We also refer to a germfree animal as "pure animal" when it is used experimentally. Thus, for the purposes of the host-contaminant problem, we have available a host which will answer the requirements of the pure culture concept and an experimental approach is open on this basis. The germfree animal may be studied as a pure animal or a pure culture of animals, or it may be brought into association experimentally with other pure cultures (microbes, etc.) in order to explain some phase of the broad problem of host-contaminant relationship.

Obtaining germfree animals is one thing, but rearing them germfree through successive generations depends on the proper apparatus and techniques. Such apparatus will not satisfy the main thesis if it is designed to merely contain a germfree animal, e.g., the hatching of a chicken in a jar. The problem of design is more basic than this and broader in implication. The basis lies in recognizing the philosophy of isolation which is a corollary of the pure culture concept. According to this philosophy, an isolate must be contained within neutral barriers and the space they enclose be expanded or contracted as needed. The environment so formed must be freed from all contaminating factors. For example, while the wall of a test tube might be considered as a barrier and the pure culture the isolate, the passage from tube to tube of the isolate, no matter how careful the technique, results in a break in the barriers because the isolate is brought into contact with a contaminated environment even though for a brief interval. This admits to possible contamination.

The design of the Reyniers Germfree System meets the requirement of the basic design because it permits complete control of the environment. The individual units (Germfree Cages) can be freed of all living contaminants by sterilization with steam under pressure. Food and water can be taken into the unit or debris removed by sterilization in situ. Air can be sterilized or filtered by devices which are a part of the unit and objects can be handled within the unit through flexible gloves or mechanical devices which are an integral part of the wall. Units can be combined in any usable numbers so that one unit can be brought to the same degree of environmental control as an adjacent unit to which it is attached, thus making it unnecessary to expose an isolated object to the external environment during a transfer or at any other time. In short, the units comprise a system for exact control of the environment whether that be microorganisms, dust, gas composition, pressure, temperature or humidity.

While we are here concerned with rearing germfree animals, it should be remarked that the system is also being used to contain dangerous pathogens in air-borne and other studies as well as for machining objects in an atmosphere free of oxygen, or running chemical studies during which manipulation is necessary in an atmosphere of known composition, pressure and humidity. In short, it is possible with this system to exactly and conveniently control the environment to a degree consistent with the experimental demands. While the shape and size of these units may change in time for convenience or some other purpose, we believe the system is adequate in principle for the purpose for which it is intended and will remain so because the principles of design around which it has been built are consistent with

the basic theories of isolation and the concept of pure culture.

Quite apart from the specific questions that might be asked about the host-contaminant relationship, and they are many, it is necessary, in our judgment, first to study the animal free from contaminants through successive generations even before it is used for experimental purposes. There are many reasons for this but the main reason is that we know scarcely anything about life under these conditions. It is necessary to establish a base line against which the action of a contaminant can be measured. Therefore, in 1930 this approach was set down as part of the long range program in Lobund Institute, contingent, of course, on being able to obtain and rear animals free from contaminants and on the development of a suitable system for controlling the environment in which this could be done with certainty. In essence this phase of the program consists in describing the germfree animal morphologically, biochemically, physiologically, and serologically, assuming of course the necessary degree of standardization with respect to health of the animal. Unless this is done experience has shown us that many mistakes will be made when the animal is used as a tool to answer specific questions about some phase of the broad problem.

If we pursue this line of thought further in relation to the problem set before us, two directions are possible: 1) backward, so to speak, in an attempt to discover and study host-contaminant relationships arising from possible prenatal contamination; and 2) forward, on the assumption that the animal contains no contaminants at all and, therefore, has no experience with them.

1. If we work backwards into the problem, there are several approaches — one through genetics, another through observations over the entire life of the animal and its progeny. There is also the direct approach in which an attempt is made to break apart any symbiotic relationships by placing stress on the animal through the use of radiation, carcinogens, etc., or to deliberately contaminate it with a known virus.

2. If we work forward in the problem on the assumption that the animal is free from contaminants at birth and after, the approaches are just as varied. The problems of nutrition and of the biochemistry involved are of considerable interest inasmuch as there is no intestinal flora to complicate the study. Quite apart from the more specific question of "intestinal synthesis" lies the possibility of studying many other things such as transport systems for chemical groups. The nutrition of the pure animal may be studied for the first time since it cannot be so investigated as a contaminated system. There are also the problems of longevity, resistance to non-viable contaminants, the many problems of physiology, inflammation, and tissue repair, to name only a few.

But quite apart from these demands is the need to establish a healthy germfree animal brought to a standard which makes it possible to use it as an experimental tool in direct approaches to specific problems. The conventional animal can never be brought to the same degree of standardization, nor can it serve in the same areas as the germfree animal with the same degree of certainty. This is so because the conventional animal cannot fill the requirements of the pure culture concept and is always a contaminated or dual biologic system in which the host through its experience with uncontrolled contaminants, presents activated systems with respect to contaminant association. Thus, we must always start in this study with the association already underway and never with a host system not activated, or ready to be experimentally activated, as is the situation with a germfree animal per se, i.e., to describe its natural history as fundamental to any challenge which might be submitted to it and always relating the study to the healthy, standard, normal, germfree individual. The pressure to apply these animals as tools in specific studies has made it difficult to adhere to these basic studies on the animal itself. Nevertheless, we think the study of the germfree animal per se is essential to the main problem of host-contaminant relationship if we are properly to evaluate experimentally induced host-contaminant associations.

Having considered briefly the need for studying the germfree animal as a pure sys-

tem, we now pass to the problem of bringing it into association with pure contaminants. The concept is simple. What we are trying to explain is the situation which exists in the normal conventional animal and this serves as a point of reference. Since the conventional animal represents an extremely complex situation, it is theoretically necessary first to describe it. This can be done in some instances with sufficient clarity as in the case of disease or the production of antibodies specific to a known antigen, but there are areas, e.g., intestinal synthesis, aging, etc. which are not so clearly describable. At any rate, experimental theory permits the factors involved to be analyzed and described before recombination under controlled conditions until the original complex is re-established. In addition, the procedure mentioned also allows synthesis of new situations not leading to a reconstitution of the natural complex, but this is another path.

In the use of the germfree animal, there is a tendency to consider the conventional animal as a control, in other words, to compare the conventional animal to the germfree animal. In some instances this is possible but in the matter of introducing a pure culture to the germfree animal the proper control is the germfree animal unassociated with a contaminant. This may seem like a minor point but again experience has shown the need for emphasizing it. The conventional animal is at any time the result of an indiscriminate experience with contaminants, whereas the germfree animal has no such experience and when a pure culture is introduced to it the effect is not experimentally the same. It is obvious that basic biologic reactions and patterns are alike in all instances, because the host, whether it is reared germfree or in a conventional manner, is endowed with the same systems and responses; it is just that in the germfree host some systems have not been activated and some responses not awakened.

The germfree animal does not show antibodies against bacteria, which are the commonest contaminants, unless it is brought into contact with bacteria. When a pure culture of bacteria is introduced, the response is specific and there are also definite tissue responses to the association. Using the germfree animal and knowing that it does not have antibodies against bacteria of any kind, that the lymphatic tissue is in general reduced, and that the wandering lymphocytes are not trained, it is possible to elicit controlled activation to the degree necessary for a step by step study of the general phenomena underlying these activities. This is not possible with the conventional animal simply because activation has started at birth.

If we attempt to associate the specific responses brought about by introducing a pure culture of bacteria into the germfree animal to resistance against infection, or to other general biologic phenomena such as aging, experiments may be set up step by step with a degree of certainty. Or, on the other hand, if the interest is in tumors or some other pathologic process, these phenomena may be studied with the knowledge that we are not confusing the picture through external contaminants which, even though they may not be specifically involved, do have an effect on the host which effect, in turn, may be related to the problem and confuse the interpretation of results.

We have tried herein to present a broad and basic approach to the host-contaminant relationship. By extending the pure culture concept to higher animals, by proper instrumentation of the principle of isolation as it involves the control of environment, and by studying the germfree animal in many species and through many generations in the absence of contaminants, it is possible systematically to set up a program for the study of host-contaminant relationship as this may be manifested both in basic and applied problems. It is obvious that in this limited space, and considering that this work has been going on since 1928, only a fragment of the results can be brought to your attention, but they will indicate the planning of the long-range program and approach. The host-contaminant relationship with its many manifestations in basic biology and medicine has a focus and that is at the point where the host and contaminants first come into contact with each other. The problem is basically that of rearing the host germfree. The methodology has been worked out and is operating. The road from this point is well marked but long.

II. *Serologic Observations in Germfree Animals**

MORRIS WAGNER

Lobund Institute, University of Notre Dame, Notre Dame, Indiana

The production of animals which can be reared and maintained in the germfree state[1-3] has presented the opportunity by which many observations regarding host-contaminant relationships can be made. This paper deals with some of the serologic reactions that have been observed in germfree and conventional laboratory-reared animals.

The existence of higher animal life in the germfree state poses the question as to whether antibody exists in animals reared devoid of microbial life. The literature is replete with reports that the serum of normal (non-injected and non-clinically infected) animals contains so-called "natural" antibodies against a variety of bacteria. These "natural" antibodies are demonstrable with bacteria which can be isolated from the animals' own digestive tract as well as with organisms which in many cases can not be demonstrated in the gastrointestinal tract nor in the environment at the time isolation procedures are attempted.

Proponents of the Hirszfeld[4] school propose that such antibody is physiologic, arising naturally through a postnatal gradual physiologic ripening of the serum proteins and is thus independent of any external stimulus. This "serogenesis" viewpoint includes the assumption that "natural" antibacterial bodies arise during the normal production of serum proteins simply through a fortuitous chemical affinity between the normally produced serum protein and certain bacterial antigens.

Others propose that this type of antibody is not natural but rather has been produced in response to external stimulation, either through contact with the specific bacterium in question or through contact with a closely related organism or antigenic substance. The external stimulation view does not necessarily negate the accidental chemical affinity idea as one means of producing antibacterial antibody since it is at least possible that some antibody may be produced in this way. However, it is generally believed that the usual way in which such antibacterial antibody arises is through some external antigenic stimulation.

Considering now the germfree animal which has spent its entire life free from viable microbial associates, it was of interest to determine whether such animals actually do possess antibacterial antibody in the absence of any prescribed procedure, such as parenteral injection, which might stimulate antibody production. Thus, if the antibacterial antibody found in the serum of conventional animals is truly a "natural" antibody, it should also appear in the germfree animal independent of any external stimulus. However, if these so-called "natural" antibodies are in reality induced via external stimuli, they probably should fail to arise in the germfree animal.

Normal conventionally-reared animals are also known to produce hemagglutinins and hemolysins reacting with certain foreign species' erythrocytes. Again there exists some controversy as to whether such hemagglutinin is physiologic or induced. Most investigators accept the theory that heterohemagglutinins and isohemagglutinins are developed under genetic control as a gradual physiologic ripening of the serum proteins and that external antigenic stimuli are not necessary in this case for their development.

However, Wiener[5] has expressed the idea that the alpha and beta isohemagglutinins in normal human blood serum arise via

* The data reported herein will be presented in greater detail in Lobund Reports No. 3 under the title "A Survey of Germfree Animals: I. The White Wyandotte Bantam and White Leghorn Chicken" by James A. Reyniers, Philip C. Trexler, Robert F. Ervin, Morris Wagner, Thomas D. Luckey and Helmut A. Gordon. These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Notre Dame, NR, 131-067.

stimulation from the external milieu by exposure to organisms, food or other materials containing compounds identical or similar to the human blood group substances A and B. If this is true, then certainly heterohemagglutinins could also arise through some similar process, e.g., horses immunized with Type XIV pneumococci give rise to anti-human RBC antibody.[6]

With regard to such heterohemagglutinins, we should be able to predict that if such antibodies are truly physiologic, then the presence or absence of viable bacteria per se in an animal should not influence formation of antibodies of this type unless the germfree state itself were to leave the animal in a serologically immature or underdeveloped state.

It should be noted that unless otherwise indicated, the following results were obtained using sera from normal chickens which were not injected or otherwise purposely treated in any way with antigenic substances. Comparisons are made between chicks raised germfree in Reyniers Germfree System and chickens reared by conventional laboratory brooder-room methods. Both germfree and conventional chicks were fed the same autoclaved semi-synthetic diet.

"Natural" Heterohemagglutinins for Rabbit Erythrocytes

Sera from chicks ranging in age from 0 to fifteen days failed to agglutinate rabbit erythrocytes. However, at the age of thirty days and older, both germfree and conventional chick sera agglutinated these erythrocytes to titers ranging from 1:4 to 1:64. There was no significant difference in titers observed in germfree vs. conventional chicks at comparable ages ranging up to approximately one year. A slight increase in titer was observed in both groups with increase in age.

The negative reaction in the young age groups was expected since it is well known that very young animals either fail to produce antibody or at best give poor responses even when treated parenterally with antigenic substances. The presence of the heterohemagglutinins in the thirty day and older germfree chicks would indicate that at least *viable* microorganisms are not responsible for heterohemagglutinin production. The question as to whether such antibody arose through "serogenesis" or by stimulation with some unknown dietary substance is still unanswered. However, the ability to eliminate the influence of viable microorganisms by use of germfree techniques offers a valuable tool for the future study of the possible role of external stimuli in the origin of such antibody.

"Natural" Antibacterial Agglutinins in Germfree and Conventional Chickens

Paracolobactrum Aerogenoides Test Tube Antigen: This organism was isolated from the feces of a conventional laboratory-reared chick and represents one of the more predominant bacteria in the digestive tract.

Young conventional chick sera failed to agglutinate this antigen in the test tube. However, conventional chicks ranging in age from thirty days to approximately one year produced titers ranging from 1:4 to 1:128. There was a gradual increase in titers observed with an increase in age.

The germfree chicks failed to agglutinate P. aerogenoides at all ages up to approximately one year.

Similar data were also obtained with a chicken strain of Escherichia coli. These results obtained with the paracolon and coliform organisms offer evidence that the so-called "natural" antibacterial antibodies are not natural but are produced as a result of exposure to external stimuli.

Micrococcus Epidermidis Test Tube Antigen: Conventional chicks 0 to fifteen days of age failed to agglutinate this organism. However at ages of thirty days to approximately one year, titers ranging from 1:4 to 1:64 were observed. A gradual increase in titer occurred with an increase in age.

The germfree chicks failed to agglutinate M. epidermidis in ages up to 170 days. However germfree chicks at the over-200-day-age level did show positive agglutination at 1:8 titer.

While M. epidermidis can be isolated from the feces of conventional chicks (which in itself might account for the presence of agglutinins in the conventional sera) it was also found that this same organism is one of the more predominant bacterial forms

that can be isolated from the diet prior to sterilization. The autoclaving of the diet fed the germfree birds destroys the viability of the bacteria intrinsic to the ingredients that make it up, as well as those introduced through handling during diet preparation. However, loss of viability after autoclaving does not preclude retention of antigenicity. The delayed appearance of anti-Micrococcus epidermidis agglutinins is probably a function of low concentration of organisms in the diet plus low antigenicity incurred either through the nature of the organism itself or as a result of the vigorous heat treatment during autoclaving.

The failure to demonstrate anti-Paracolobactrum aerogenoides or anti-Escherichia coli agglutinins in germfree chicks up to one year of age was correlated to the failure to demonstrate such organisms in the diet. We have never been able to detect coliform organisms in periodic tests on the preautoclaved ration.

However, the presence of dead M. epidermidis in the diet may account for the production of agglutinins in the older germfree chicks by a process analogous to immunization with oral vaccines.

Antibody Response to Large Numbers of Dietary Organisms: In the case of Micrococcus epidermidis, we were dealing with dietary organisms present in small numbers as adventitious "impurities" of the other dietary ingredients.

The following are isolated observations on chicks fed large numbers of dead bacteria which were purposely incorporated in the ration. The source of bacteria was from "BY-21," a fermentation product of the Commercial Solvents Corporation. It was incorporated in the diet at a 2 per cent level. Direct microscopic examination of the diet showed large numbers of bacteria to be present. The antigen used for testing the presence of agglutinins was prepared from a bacillus species isolated from the BY-21 before autoclaving.

Two germfree chicks receiving large numbers of dead bacteria in the BY-21 diet produced agglutinins to titers of 1:16 and 1:32 against the BY-21 bacillus when observed at ages of forty-two and sixty-nine days respectively (well prior to the 200 day Micrococcus epidermidis agglutinins previously referred to). The anti-E. coli and anti-M. epidermidis titers for these sera were negative. A germfree chick not receiving dietary BY-21 failed to agglutinate the BY-21 antigen at age thirty-nine days.

The Response of Germfree and Conventional Chicks to Parenteral Injection of Antigen

The question is often asked, "How will the germfree animal, which has had no immunologic experience with living microorganisms, react to non-viable antigenic substances when introduced by parenteral injection?" Germfree and conventional chickens were given intravenous injections of Salmonella pullorum bacterin or sterile beef serum.

In this experiment, no attempt was made to produce maximum titers in these animals since it was feared that over-stimulation might overcome or mask initial differences in the two groups. The reactions recorded here were in response to three small intravenous injections given on alternate days.

The germfree and conventional chicks reacted almost identically to parenteral injection, producing average agglutinin titers of 1:128 against the Salmonella pullorum and average precipitin titers of 1:4096 for beef serum. The non-treated germfree and conventional chick sera gave negative reactions for anti-Salmonella pullorum agglutinin and anti-beef serum precipitin. It remains to be seen whether single small dose injections may show more subtle differences. At this stage, it appears that the germfree state has not handicapped the antibody-producing apparatus.

Summing up, it was shown that germfree chickens produce heterohemagglutinins in time and titer quite comparable to conventional chicks. It has also been shown that antibacterial antibody apparently arises in response to external stimuli. Conventional chicks, harboring coliform bacteria as part of the intestinal flora, produce agglutinins which react with these intestinal isolates. Germfree chicks, on the other hand, were not exposed to viable coliforms nor could any evidence be found that they were exposed to dead coliforms in the diet. Germ-

free chicks failed to develop anti-coliform antibody.

That germfree chicks can produce antibacterial agglutinins in response to external stimuli was demonstrated by the fact that Micrococcus epidermidis could be isolated from the diet prior to sterilization and that germfree chicks fed such sterile diets eventually produced agglutinins against this organism. It was also shown with the BY-21 diet that feeding large numbers of dead bacteria shortened the time at which antibacterial antibody could be detected in germfree chicks.

Finally, it was shown that intravenous injection of antigenic substances into germfree and conventional chicks resulted in the production of comparable antibody titers in the two groups.

REFERENCES

1. Reyniers, J. A. and Trexler, P. C. The germfree technique and its application to rearing animals free from contamination, in *Microsurgical and germ-free techniques*, Springfield, Ill., C. C. Thomas, 1943, pp. 144-163.
2. Reyniers, J. A., Trexler, P. C. and Ervin, R. F. Rearing germ-free albino rats, *Lobund Reports*, No. 1, 1946.
3. Reyniers, J. A., Trexler, P. C., Ervin, R. F., Wagner, M., Luckey, T. D. and Gordon, H. A. Rearing germ-free chickens, *Lobund Reports*, No. 2, 1949.
4. Hirszfeld, L. *Konstitutionsserologie und Blutgruppenforschung*. Berlin, Springer, 1928.
5. Wiener, A. S. Origin of naturally occurring hemagglutinins and hemolysins; a review, *J. Immunol.* 66:287-95, 1951.
6. Finland, M. and Curnen, E. C. Agglutinins for human erythrocytes in Type XIV antipneumococcic horse serums, *Science* 87:417-18, 1938.

III. *Morphologic Characterization of Germfree Life**

HELMUT A. GORDON

Lobund Institute, University of Notre Dame, Notre Dame, Indiana

The aim of this study was to characterize the germfree animal with data concerning growth, organologic status, reproduction and other general criteria of health. Subsequently, the role of the lymphocyte was taken up in some morphologic and functional detail and from this viewpoint a comparison was drawn between the germfree and conventional animal.

The materials forming the basis of this report were chickens and rats distributed in groups of mixed sexes, aged from the newborn to one year.

Morphological Description of the Germfree Animal: On first approximation, it can be stated that higher organisms living in the absence of germs do not change in macroscopic appearance. Growth rate, red cell and hemoglobin concentration of the blood are close to normal, or somewhat higher in the germfree. However, this difference never reaches the degree of statistical significance. The germfree animal matures and begins its cycle of reproduction at the same age as, or a little earlier than, the normal conventionals. At present there are rats in Lobund's germfree colony which represent the eighth generation after the initial litter born by cesarean operation from a conventional mother animal. In respect to aging, our observations are only spotty; however, they permit us to state that the germfree animal lives at least as long as the conventionals of the stock colony. Thus, from the viewpoint of general health, the germfree animal appears normal, when measured with

* Some of the data reported herein will be presented in greater detail in Lobund Reports No. 3 under the title "A Survey of Germfree Animals: I. The White Wyandotte Bantam and White Leghorn Chicken" by James A. Reyniers, Philip C. Trexler, Robert F. Ervin, Morris Wagner, Thomas D. Luckey and Helmut A. Gordon. These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Notre Dame, NR, 131-067.

the yardstick of conventional laboratory standards.

However, a systematic survey of our animals has also revealed the existence of some vitally irrelevant, but biologically essential, differences between the germfree and the conventional. These differences can be briefly summarized. Organs which in normal conditions are in direct contact with a bacterial flora, e.g., the gastrointestinal tract, weigh significantly less in the germfree than in the conventionals (expressed in weight per 100 gm. body weight). This characteristic is also shared by the lymph follicles and nodes which are dispersed in or adjacent to the intestinal canal, such as: the Peyer patches, mesenteric lymph nodes and notably the ileocecal tonsil of the chicken. Other organs of the germfree animal which in the contaminated animal have no flora-contact, show identical weight conditions, duplicating the normal controls. This seems to apply also to the more remote lymphocyte-bearing organs, such as the spleen. Thus, in this comparative study between the germfree and the conventional, there have been established two clearly different groups of organs. One group faces the exterior and has a significantly reduced weight in the germfree; the other, which is harbored in the interior of the organism, shows no difference in the two animal categories.

In an effort to interpret this difference, only partial answers can be offered. It has been established that the lesser weight of the external environment organs of the germfree is not paralleled by dry-weight differences, thus ruling out the possibility of a difference in hydration. Similarly, it has been found that the mass of lymphocytes, though actually reduced in these organs of the germfree animal, could not account for the rather substantial weight-deficit observed. Preliminary evidence shows that certain differences in the amount of connective tissue are mainly responsible for this phenomenon.

At this point an additional detail should be emphasized. One of our previous statements implied that in terms of endocrine organ weights there is no difference between germfree and conventionals. Indeed, all our animals appeared endocrinologically normal. This fact, combined with the reduced weight of some lymphopoietic organs in the germfree, merited special attention, particularly in view of some current theories concerning the relationship between the endocrines and lymphocytic mobilization. Therefore, in order to contribute additional evidence to this question, parallel determinations of adrenal cholesterol and ascorbic acid were run, in order to gauge, presumably, the functional status of the adrenal cortex. The results showed that there was no difference from this viewpoint between these two animal categories. It is believed that this evidence of endocrine stability is an added support in proving the normalcy of the germfree animal.

Another interesting difference between the germfree and the conventional animals concerns the degree of variability in some of their morphologic and functional characteristics. A comparison of the coefficients of variability of organ weights in the two animal categories clearly demonstrates that in most organs of the external environment, the germfree animal shows a lower scattering than the conventional, as exemplified in the case of the small intestine and the ileocecal tonsil. In respect to the organs of the internal environment, there is no difference in scattering between the two groups. At present only a speculative explanation is offered for this evidence. It is plausible that organs unexposed to the flora-stimulus, such as in the germfree, should respond with a higher degree of uniformity. Experimental details of this question are presently under elaboration.

Observations Concerning the Lymphocyte in Germfree Animals: The discussion concerning the role of the lymphocyte in this field of investigation is best introduced by way of the previously mentioned, greatly reduced weight of the ileocecal tonsil in the germfree chicken. For example, between the sixty day old germfree and conventional White Wyandotte Bantam chicken the ratio in the weight of this organ is approximately 1 to 2. With increasing age, this ratio can reach even 1 to 3 or more. Histologic observation has shown that the reduced weight of this organ in the germfree animal is paralleled by a considerable reduction in its lym-

phocyte content. Following this lead the concentration of lymphocytes was determined in various organs where this was feasible and appeared to be of some importance. Our results showed, for example, that the ileocecal tonsil, an organ of the external environment, shows a drastically lower lymphocytic content in the germfree (germfree-conventional ratio 1:10). The spleen, a representative of the organs of internal environment, failed to demonstrate any differences in this respect. The blood, which probably draws its lymphocyte supply from both the organs of external and internal environment, occupies a mid-position between these two extremes (germfree-conventional ratio, 1:2). The combined results unquestionably demonstrate the importance of the bacterial flora in determining the size of the lymphocytic stock of exposed organs.

In an effort to elucidate these findings from the functional viewpoint only preliminary observations can be offered. Actually, a common treatment of both rat and chicken lymphocyte data appears permissible for this purpose because the basic response of these cells to bacterial stimulation always showed an identical pattern in spite of the fact that two such distant and unrelated species were involved.

The first question investigated was the antigenic sensitization of the lymphocyte. Is it possible to demonstrate a parallelism between the spectacular lymphopoietic stimulation of the ileocecal tonsil and a sensitization of the involved lymphocytes against intestinal bacterial antigens? If so, is this sensitization absent in the instances when there is no direct flora-contact, such as is the case in all lymphopoietic centers of the germfree or in the remote internal environment centers of the conventional animal? Using the lymphocytolysis technique of Favour,[1] lymphocytes of various origins have been tested in vitro against a number of intestinal and other antigens. So far, the evidence is not conclusive and therefore only the progress of the experiments is reported herein.

Our work has revealed some more detail in the studies concerning the production and utilization (release and removal) of the lymphocytes by various organs. These experiments were performed mainly in rats and should be regarded as preliminary tests only. The procedure consisted of taking blood samples from the vein of different organs; simultaneously samples were secured from the arterial system. Thus, the concentration of white blood elements was established before and after the blood flowed through the organ. This indicated the positive or negative contribution which occurred in the course of the passage. The values have been corrected for arterio-venous hemoconcentration but not, as yet, for flow. Generally, the procedure was similar to that described by Ambrus and others.[2]

Blood samples taken from the upper, mid and lower small intestine of the conventional rat indicated that lymphocytes were being removed from the blood at all points of this organ. As far as the number of the lymphocytes removed from the blood was concerned, an inverted relationship seemed to exist between this value and the bacterial content of the intestine: i.e., the lower the segment of the intestine, the less lymphocytes were removed from the blood. This finding appears to be somewhat paradoxical if it is accepted that the lymphocyte participates and is used up in the defense against bacteria. However, the paradox is dissolved if it is considered that the blood, as a conveyor of lymphocytes, is gradually relieved by the increasing lymphopoietic resources of the lower gut. Splenic blood showed that this organ, as is well known, is a major source of lymphocytes in the conventional animal.

A test case for the correctness of these data and conclusions is offered by the germfree animal, where 1) no bacteria are present in the intestinal canal, 2) presumably no lymphocytes need to be removed from the blood for defense purposes and finally, 3) the lymphopoietic centers in the lower gut are functioning only at a certain idling speed. The results show the following pattern. The upper part of the small intestine which presumably produces and utilizes only few lymphocytes, affects only little the blood's arterial lymphocytic level during the course of the passage. In the lower portions of the intestine, an increasing amount of lymphocytes was found in the venous blood and it is conjectured that this is the result

of the reduced, but still present, lymphopoietic activity of the lower gut in germfree conditions. The spleen of the germfree animal appears to release significantly fewer lymphocytes than that of the conventional animal. This observation is in apparent contradiction with a previous statement in which it was maintained that in this organ there is no difference in lymphocytic stock between the two animal categories. The explanation of this discrepancy probably is that stock and production of a cell type, in a given organ, do not necessarily go hand in hand.

Thus far, this paper has dealt only with animals that were fully adapted to their bacterial or bacterium-free environment. Some data have been obtained, however, on the changes in lymphopoietic status which accompany the transition from germfree to contaminated life. An illustration of this is the lymphocyte production of the spleen as it was observed in one germfree and in three other ex-germfree rats which were sacrificed one, two and three weeks after a gross bacterial contamination. A complement of the picture is given by the concomitant data concerning one conventional rat. The net difference between the germfree and the conventional, i.e., the initial and the terminal state in this case, is in the threefold production of spleen-lymphocytes. During the three weeks of transition, however, the spleen-lymphocyte output was as follows: 1st week 15-, 2nd week 30-, and 3rd week 7-fold increase over the original germfree value. These data appear to be a good general example of the progress of adaptative phenomena. It is interesting to note that the appearance of antibodies usually coincides with the subsiding of this spectacular lymphocytic response.

REFERENCES

1. Favour, C. B. Lytic effect of bacterial products on lymphocytes of tuberculous animals, *Proc. Soc. exp. Biol. Med. 65:269-72*, 1947.
2. Ambrus, J. L., Ambrus, C. M., Johnson, G. C., Packman, E. W., Back, N., Chernik, W. and Harrisson, J. W. E. The removal and release of white blood cells by extramedullary organs. *Abstracts of the 19th International Physiological Congress*, Montreal, 1953.

Herald Square, N. Y

Parkchester, Bronx

Jamaica, Queens

White Plains, N. Y

Flatbush, Brooklyn

MACY'S PRESCRIPTION DEPARTMENT

Macy's is accurate. Our Prescription Department is a modern, sanitary laboratory, staffed by competent pharmacists. All of our completed prescriptions are carefully checked by supervising pharmacists. Our stock of ingredients is wide and reliable; it includes not only standard drugs, but also new and scarce products. And our prices are low.

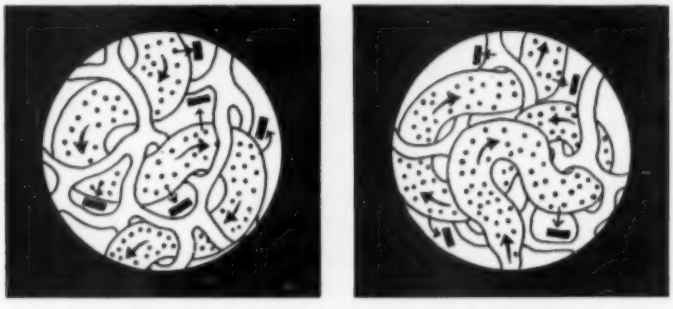
No wonder so many physicians prefer Macy's Prescription Department.

MICTINE*—THE NEW ORAL DIURETIC

Searle MICTINE Provides Effective Oral, Non-Mercurial Diuresis

The result of many years of research, Mictine, brand of aminometramide, supplies a long-felt need for an improved oral diuretic. Mictine, 1-allyl-3-ethyl-6-aminotetrahydropyrimidinedione, is not a mercurial, xanthine or sulfonamide.

Effectiveness: Every method for measuring the diuretic effect in man now available, including precise human bioassay studies, without exception demonstrated that Mictine is an effective oral diuretic, and these studies show that approximately 70 per cent of *unselected* edematous patients treated with Mictine by mouth respond with a satisfactory diuresis.



Mictine is believed to act by the selective inhibition of the reabsorption of sodium ions. Thus, the resulting diuresis is characterized by increased quantities of sodium ions and water.

Well-Tolerated: There are no known contraindications to Mictine, even in the presence of hepatic or renal damage, and there is no risk of acidosis. On high dosage, Mictine causes some side effects in some patients but on three tablets daily these side effects (anorexia and nausea, rarely vomiting, diarrhea or headache) are minimal or absent.

Indications: Mictine is useful primarily in the *maintenance* of an edema-free state and in the *initial and continuing* control of patients in mild congestive failure. Mictine may be used also for *initial and continuing* diuresis in *more severe* congestive states, particularly when mercurial diuretics are contraindicated.

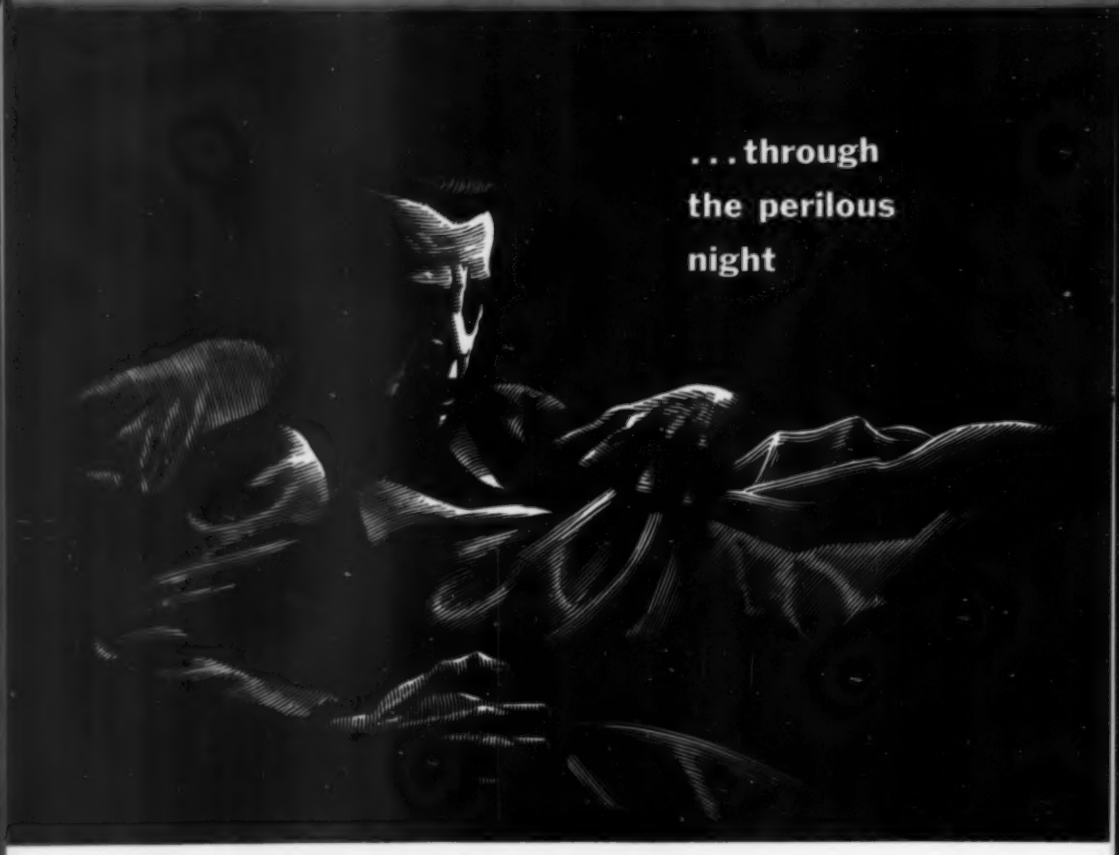
Administration: The usual dosage for the average patient is one to four tablets daily with meals, in divided doses on an interrupted schedule. An interrupted dosage schedule may be accomplished by giving the drug on alternate days or for three consecutive days and then omitting it for four days.

For severe congestive states the dosage is four to six tablets daily with meals, in divided doses on interrupted schedules similar to those already mentioned.

Supplied: Uncoated tablets of 200 mg.

**Trademark of G. D. Searle & Co.*

SEARLE



You can prevent attacks in angina pectoris

Fear is a faithful companion. In angina pectoris, particularly, many patients live in constant dread of recurrent attacks.

Prophylaxis with Peritrate, a long-acting coronary vasodilator, offers new security in a majority of such cases. A single dose affords protection for as long as 4 to 5 hours, compared to 30 minutes or less with nitroglycerin.

Different investigators[1-3] observed that 80% of their patients responded to Peritrate therapy with fewer, less severe attacks . . . reduced nitroglycerin dependence . . . improved EKG's.

A variety of convenient dosage forms now extends these benefits. Peritrate Delayed Action tablets (10 mg.), taken with the regular bedtime dose of Peritrate (plain) help allay the fear of nighttime attacks. Adapted to the recommended daily dosage of 40-80 mg., Peritrate is available in 10 mg. and 20 mg. tablets. And when added sedation is indicated, you can prescribe Peritrate (10 mg.) with Phenobarbital (15 mg.).

1. Winsor, T., and Humphreys, P.: Angiology *3*:1 (Feb.) 1952. 2. Plotz, M.: New York State J. Med. *52*:2012 (Aug. 15) 1952. 3. Dailheu-Geoffroy, P.: L'Ouest-Médical, vol. 3 (July) 1950.

Peritrate®

tetranitrate

(BRAND OF PENTAERYTHRITOL TETRANITRATE)

WARNER-CHILCOTT

NEW! 'Tetrazets'

BACITRACIN-TYROTHRICIN-NEOMYCIN-BENZOCAINE TROCHES

broader attack to overcome minor throat irritations

MAJOR ADVANTAGES: Combines 3 antibiotics to fight both gram-positive and gram-negative bacteria. Benzocaine included for soothing effect. Little danger of sensitization.

'TETRAZETS' quickly relieve minor mouth and throat irritations

It's new—a single troche containing 3 potent antibiotics (bacitracin, tyrothricin, neomycin) to combat afebrile oral infections.

'TETRAZETS' offer you the ideal topical treatment of minor irritations of the oral cavity.

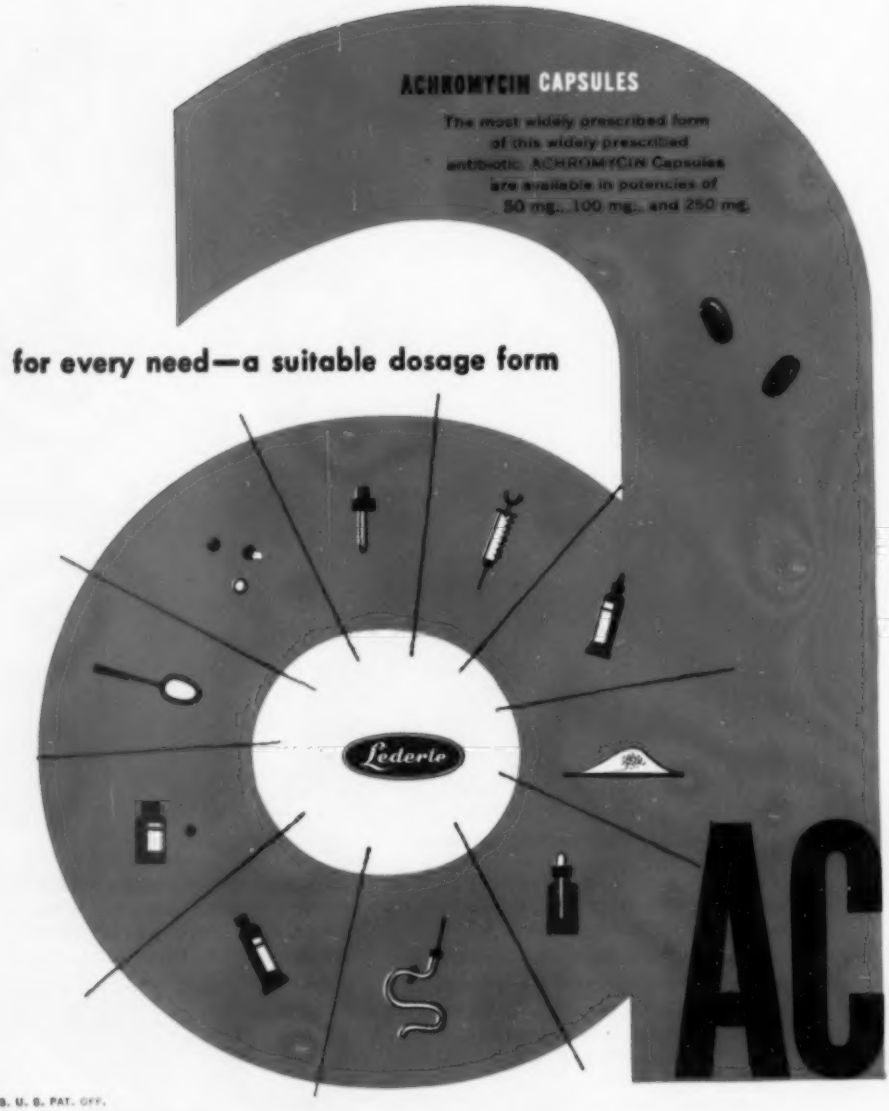
In deep-seated infections, such as Vincent's infection, tonsillitis and streptococcus sore throat, 'TETRAZETS' may be used as an adjuvant to parenteral antibiotics.

Before and after tonsillectomies, 'TETRAZETS' help combat secondary invaders.

Supplied: In vials of 12. Each 'TETRAZETS' troche contains 50 units of zinc bacitracin, 1 *mg.* tyrothricin, 5 *mg.* neomycin sulfate with 5 *mg.* benzocaine.

SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.


ACHROMYCIN CAPSULES
The most widely prescribed form of this widely prescribed antibiotic. ACHROMYCIN Capsules are available in potencies of 50 mg., 100 mg., and 250 mg.
for every need—a suitable dosage form
Lederle
AC

*REG. U. S. PAT. OFF.

Other Dosage Forms:

TABLETS: 50, 100, and 250 mg.

PEDIATRIC DROPS (Cherry Flavor): 100 mg. per cc. (approx. 5 mg. per drop), 10 cc. bottle

ORAL SUSPENSION (Cherry Flavor): 250 mg. per teaspoonful (5 cc.), 1 oz. bottle

SPERSOIDS* Dispersible Powder (Chocolate Flavor): 50 mg. per rounded teaspoonful (3 Gm.), 12 and 25 dose bottle

SOLUBLE TABLETS: 50 mg.

INTRAVENOUS: vials of 100, 250, and 500 mg.

INTRAMUSCULAR: vial of 100 mg.

OINTMENT (3%): ½ and 1 oz. tubes

OPHTHALMIC OINTMENT (1%): ⅛ oz. tubes

OPHTHALMIC SOLUTION: vial of 25 mg. with sterilized dropper vial

EAR SOLUTION (0.5%): 10 cc. dropper bottle

SYRUP (Cherry Flavor): 125 mg. per teaspoonful (5 cc.), 2 oz. bottle

In any of its numerous dosage forms, ACHROMYCIN is a potent weapon to combat a variety of infections. This true broad-spectrum antibiotic has proved effective in controlling Gram-negative and Gram-positive bacteria, rickettsia, spirochetes, and certain viruses and protozoa.

And ACHROMYCIN is unusually kind to the patient! Extensive use, in children and adults, has shown it to be practically free of untoward side reactions. ACHROMYCIN diffuses rapidly in body tissues and fluids, is readily soluble, and remains stable for 24 hours in solution.

When a broad-spectrum antibiotic is indicated, remember—for your convenience, for the patient's comfort, there's a particular dosage form of ACHROMYCIN to fill the need!

LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY PEARL RIVER, NEW YORK

Now available as Syrup—ready-mixed!

*HROMYCIN

TETRACYCLINE LEDERLE

Pure as sunlight

Upjohn

Sex hormones— only one injection per month:

Depo-Estradiol

Trademark, Reg. U. S. Pat. Off.

CYCLOPENTYLPROPIONATE

Each cc. contains:

Estradiol, 17-Cyclopentylpropionate	1 mg. or 5 mg.
Chlorobutanol	5 mg.
Cottonseed Oil	q.s.

1 mg./cc. strength in 10 cc. vials
5 mg./cc. strength in 5 cc. vials

Depo-Testosterone

Trademark, Reg. U. S. Pat. Off.

CYCLOPENTYLPROPIONATE

Each cc. contains:

Testosterone Cyclopentylpropionate	50 mg. or 100 mg.
Chlorobutanol	5 mg.
Cottonseed Oil	q.s.

50 mg./cc. strength in 10 cc. vials
100 mg./cc. strength in 1 cc. and 10 cc. vials

Depo-Testadiol

Trademark, Reg. U. S. Pat. Off.

Each cc. contains:

Testosterone Cyclopentylpropionate	50 mg.
Estradiol, 17-Cyclopentylpropionate	2 mg.
Chlorobutanol	5 mg.
Cottonseed Oil	q.s.

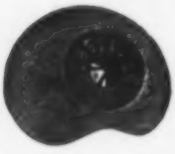
10 cc. vials

The Upjohn Company, Kalamazoo, Michigan

"Premarin" relieves menopausal symptoms with virtually no side effects, and imparts a highly gratifying "sense of well-being."

"Premarin" — Conjugated Estrogens (equine)

PLASTIC AND GLASS EYES



MAGER & GOUGELMAN, Inc.

OVER A CENTURY OF SERVICE • 1851-1952

- PRIVATE FITTINGS
- PERSONAL ATTENTION
- EXPERIENCE & SKILL

510 MADISON AVE., N. Y. 22
Telephone PLaza 5-3756

when proper or corrective shoes are indicated . . .

prescribe **PEDIFORMES**

IN STOCK:

- straight last shoes
- inflare last shoes
- outflare last shoes
- club foot shoes
- flat foot shoes
- childen's shoes with Thomas Heel and long counter

You are assured alterations and fittings as you prescribe because of our interest, and experience gained in serving the medical profession for more than 40 years.

Pediforme

34 West 36th STREET
New York City
Wisconsin 7-2534

Other Shops in: Brooklyn • Flatbush • Hempstead
New Rochelle • Hackensack

The long and short of Bentyl's relief of nervous gut

Clinicians[1,2] prove Bentyl is long on effective relief... short on unwanted side effects including blurred vision and dry mouth.

1. McHardy and Browne: Sou. Med. J. 45:1139, 1952. 2. Lorber and Shay: Fed. Proc. 12:90, 1952.

Complete Bentyl bibliography on request.

BENTYL

(Dicyclomine Hydrochloride)

another exclusive development of Merrell research

Rx INFORMATION

BENTYL

Bentyl affords direct (musculotropic) and indirect (neurotropic) spasmolytic action. Bentyl provides complete and comfortable relief in smooth muscle spasm, particularly in functional G.I. disorders, in irritable colon, pylorospasm, biliary tract dysfunction and spastic constipation.

Composition: Each capsule or teaspoonful (5 cc.) contains 10 mg. of Bentyl (dicyclomine hydrochloride).
Bentyl with Phenobarbital adds 15 mg. of phenobarbital to the preceding formula.

Dosage: *Adults,* 2 capsules or 2 teaspoonfuls of syrup, three times daily, before or after meals. If necessary, repeat dose at bedtime. *In Infant Colic,* ½ to 1 teaspoonful, ten to fifteen minutes before feeding.

Supplied: Bentyl — In bottles of 100 and 500 blue capsules, and Bentyl Syrup in pint and gallon bottles. Bentyl with Phenobarbital — In bottles of 100 and 500 blue-and-white capsules, and Bentyl Syrup in pint and gallon bottles.

Merrell
Since 1828

PIONEER IN MEDICINE FOR OVER 125 YEARS

THE WM. S. MERRELL COMPANY New York · CINCINNATI · St. Thomas, Ontario

agents of choice

for the treatment of ***pneumonia*** *and other respiratory tract infections*

Terramycin®

BRAND OF OXYTETRACYCLINE

For (ESTABLISHED) broad-spectrum antibiotic therapy—supplied in convenient Capsules, Tablets (sugar coated), Oral Suspension (raspberry flavored), Pediatric Drops (raspberry flavored), Intramuscular, Intravenous, Ophthalmics, Ointment and other topical forms.

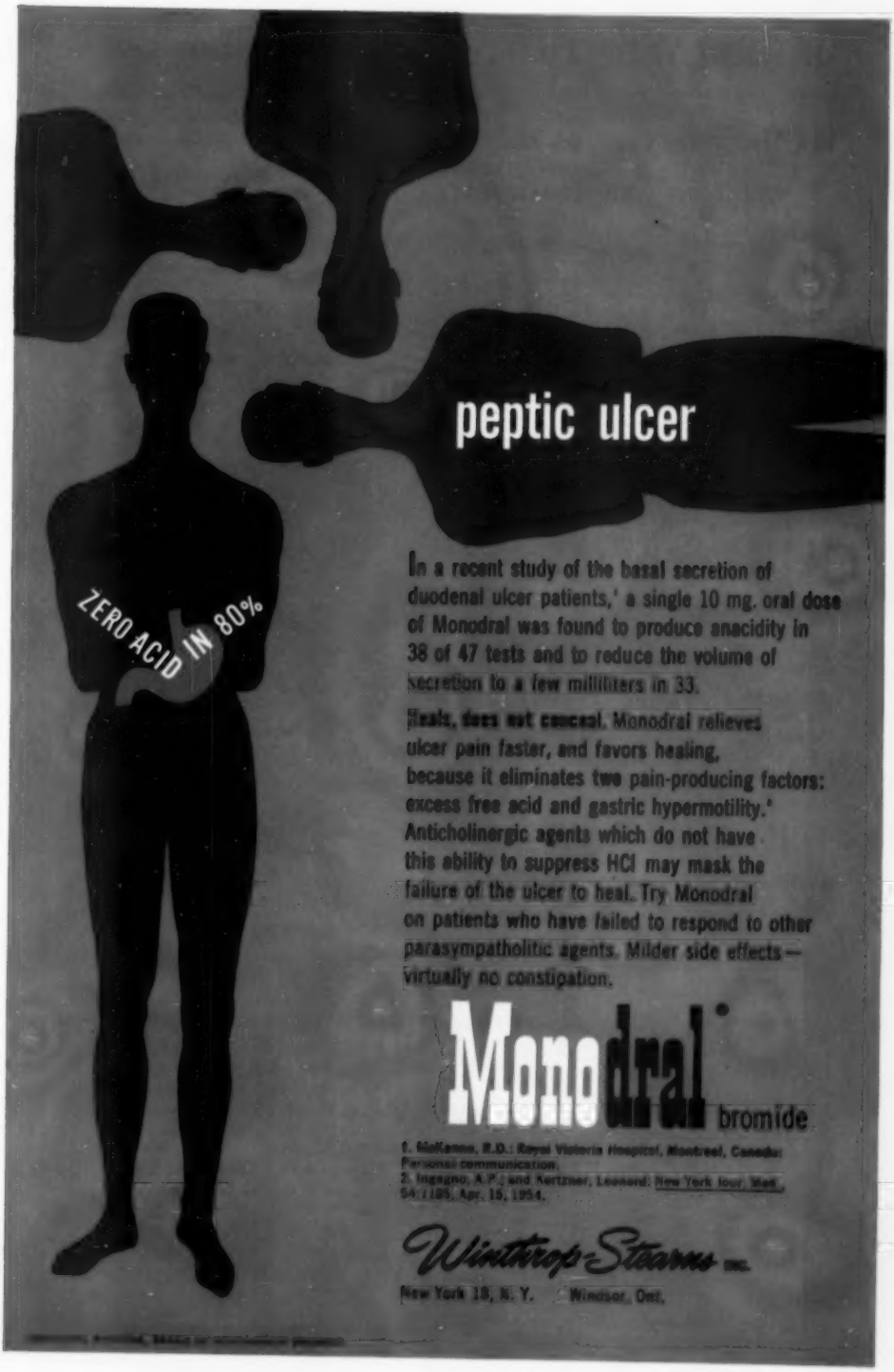
Tetracyn®

BRAND OF TETRACYCLINE

For the (NEWEST) broad-spectrum antibiotic therapy—supplied in convenient Capsules, Tablets (sugar coated), Oral Suspension (chocolate flavored), Pediatric Drops (banana flavored), Intramuscular, Intravenous, Ophthalmic and Ointment.

Both discovered by Pfizer *world's largest producer of antibiotics*

PFIZER LABORATORIES, Brooklyn 6, N. Y.
Division, Chas. Pfizer & Co., Inc.


peptic ulcer
ZERO ACID IN 80%
In a recent study of the basal secretion of duodenal ulcer patients,' a single 10 mg. oral dose of Monodral was found to produce anacidity in 38 of 47 tests and to reduce the volume of secretion to a few milliliters in 33.
Heals, does not conceal. Monodral relieves ulcer pain faster, and favors healing, because it eliminates two pain-producing factors: excess free acid and gastric hypermotility.' Anticholinergic agents which do not have this ability to suppress HCl may mask the failure of the ulcer to heal. Try Monodral on patients who have failed to respond to other parasympatholitic agents. Milder side effects—virtually no constipation.
Monodral® bromide
1. McKenna, R.D.: Royal Victoria Hospital, Montreal, Canada: Personal communication.
2. Ingegno, A.P., and Nertzer, Leonard: New York Jour. Med., 54:1185, Apr. 15, 1954.
Winthrop-Stearns INC.
New York 18, N. Y. Windsor, Ont.

CHARLES B. TOWNS HOSPITAL *Established 1901*

FOR THE TREATMENT OF ALCOHOLISM

NARCOTIC AND BARBITURATE

ADDICTIONS EXCLUSIVELY

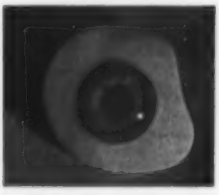
Complete Medical & Psychiatric Treatment at predetermined cost. Privacy of patient is assured—if desired.

Literature on Request

Edward B. Towns, Director

293 Central Park West, N. Y. 24, N. Y
SChuyler 4-0770

Member American Hospital Association



SATISFACTION GUARANTEED

Specialists in Plastic and Glass Artificial Human Eyes Exclusively

Referred Cases Carefully Attended

FRIED & KOHLER, Inc.

665 FIFTH AVENUE near 53rd Street
NEW YORK, N. Y. Tel. ELdorado 5-1970

For acidosis due to anesthesia — edema

KALAK

Counter-Acts

ANTI-BIOTIC REACTIONS

. . . . KALAK is a non-laxative, alkaline diuretic buffer — side reactions from aureomycin — terramycin — sulfas — penicillin are reduced through the use of KALAK — KALAK contains only those salts *NORMALLY* present in plasma. . . . IT IS BASIC!

KALAK WATER CO.
of NEW YORK, Inc.
90 West St., New York 6, N. Y.

For acidosis due to nausea — in nephritis

SEDATION AND EUPHORIA FOR NERVOUS, IRRITABLE PATIENTS

Use **VALERIANETS-DISPERT**

Reg. U. S. Pat. Off.

Each Chocolate Coated Tablet Contains Ext. Valerian (highly concentrated) 0.06 Gm. dispergentized finely subdivided for maximum efficiency

TASTELESS, ODORLESS, NON-DEPRESSANT SEDATIVE and EUPHORIC

VALERIANETS-DISPERT are indicated in cases of nervous excitement and exhaustion, anxiety and depressive states, cardiac and gastrointestinal neuroses, menopausal and menstrual molimena, insomnia.

Dose: 1 or 2 tablets t.i.d. — Bottle of 50 and of 100 tablets

At All Prescription Pharmacies

STANDARD PHARMACEUTICAL CO., INC., 253 West 26th St., New York

For Intestinal Dysfunction
NUCARPON®
Each tablet coat: Extract of Rhubarb, Senna, Precip. Sulfur, Peppermint Oil, Fennel Oil in activated charcoal base.

For making Burow's Solution U.S.P. XIV
WET DRESSING Use
PRESTO-BORO®
(Aluminum Sulfate and Calcium Acetate)
POWDER IN ENVELOPES — TABLETS —
For treatment of Swellings, Inflammations, Sprains

For Pulmonary Conditions
TRANSPULMIN®
5% solution Quinine with 2½% Camphor for intramuscular Injection.

BETTER TOLERATED SALICYLATE THERAPY

For Headache, Neuralgia, Minor Aches and Pains
Give BUFFERIN® because...

1. It gives fast pain relief—acts twice as fast as aspirin.[1]
2. Even large doses seldom cause gastric upsets.[2]

For Arthritis—and Other Rheumatic Disorders
Give BUFFERIN because...

1. It provides effective, better-tolerated relief of pain.
2. There were no gastric upsets with BUFFERIN in 70% of hospitalized arthritic patients who couldn't tolerate aspirin.[3] This is an important finding, for arthritics are 3 to 9 times as susceptible to gastric upsets with straight aspirin as the general population.[4]
3. The antacids in BUFFERIN do not lower the blood salicylate levels as sodium bicarbonate does.[4]

BUFFERIN contains acetylsalicylic acid (5 gr. per tablet), for prompt analgesia, plus magnesium carbonate and aluminum glycinate.

Available—bottles of 12, 36, 60 and 100 tablets.

References: 1. J. Am. Pharm. Assoc., Sc. Ed. 39:21 (Jan.) 1950. 2. Ind. Med. 20:480 (Oct.) 1951. 3. In Press. 4. J.A.M.A. 141:124 (Sept. 10) 1949.

WHENEVER SALICYLATE THERAPY IS INDICATED GIVE BUFFERIN® Acts Twice as Fast as Aspirin Does Not Upset the Stomach

BRISTOL-MYERS CO.,
19 W. 50 St., New York 20, N. Y.

a pain-free stomach—
quiet intestines—
for your nervous patients

'TRICOLOID'*

quickly blocks the nervous impulses which cause gastrointestinal symptoms

causes negligible dryness of the mouth or other side effect.

*'TRICOLOID' brand Tricyclamol 50 mg. Compressed, sugar-coated
Bottles of 100

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe 7, New York

broad spectrum antibiotic of choice

STECLIN

SQUIBB TETRACYCLINE

reaches higher levels in the urine than its analogues

crosses the intact meningeal barrier more readily than its analogues

produces higher blood levels than its analogues

fewer gastrointestinal side effects than its analogues

Minimum adult dose: 250 mg. q.i.d.

Capsules: 250 mg., bottles of 16 and 100.
50 and 100 mg., bottles of 25 and 100.

Suspension: 1 oz. bottles, supplied with dropper calibrated at 1 cc.
Each 5 cc. teaspoonful contains the equivalent of 250 mg.
of tetracycline hydrochloride.

SQUIBB

"STECLIN" IS A SQUIBB TRADEMARK

Hoeber-Harper H|H *announces . . .*

THREE IMPORTANT *new* BOOKS

BAKER'S:

Clinical Neurology

In 3 Volumes. A complete source of today's knowledge of diagnosis and treatment—including not only the more common neurologic disturbances but also the rarer syndromes and the ramifications of neurology in the fields of internal medicine, pediatrics, endocrinology, psychiatry, ophthalmology and otolaryngology. While the first objective is to organize for practical use the known facts of neurology, much new material is published here for the first time.

65 Authorities. Edited by A. B. BAKER, *M.D., Prof. and Dir., Div. of Neurology, Univ. of Minn. Med. Sch., 2100 pp., liberally illustrated, 3 volumes $60.00.*

WEBER'S:

The Thyroid

This interdisciplinary approach to the thyroid gland provides the first complete guide to thyroid functions, disorders and relationships. From the fundamental biochemistry to the details of treatment, both medical and surgical, including laboratory technics as well as the latest radioactive materials, you will find today's best knowledge on any question you may look up. Each topic is presented by an authority; yet, because the book is so carefully planned and edited, it reads like a consecutive account by a single authority.

53 Authors. Edited by SIDNEY C. WEBER, *M.D., Assoc. Prof. of Clinical Medicine, Columbia Univ. Coll. of Physicians and Surgeons. Approx. 800 pp. In Press.*

DUNHAM'S:

Premature Infants

2nd Edition

A Manual for Physicians. The basic source of information on prematurity and guide to care of the premature infant. Part I deals with general considerations; criteria for prematurity; incidence, causes and prevention of premature birth; growth and development of premature infants. Part II takes up the physiologic handicaps of premature infants, their problems and their care; congenital and acquired conditions that tend to affect them adversely. Part III outlines public health aspects of prematurity, including death rates and causes of death.

EDITH C. DUNHAM, *M.D., Children's Bureau. In Press.*

All Hoeber-Harper Books Sent On Approval

PAUL B. HOEBER, *Inc., Publisher,* 49 East 33rd St., N.Y. 16

MEDICAL BOOK DEPARTMENT OF HARPER & BROTHERS